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Original Article

SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NOVEL 2-HYDRAZONO-2(3*H*)-THIAZOLE DERIVATIVES DERIVED FROM 4-(3-CHLOROPHENYL)-3-THIOSEMICARBAZIDE

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ABSTRACT

Objective: A series of substituted 2-hydrazono-2(3*H*)-thiazole derivatives were synthesized from 4-(3-chlorophenyl)-3-thiosemicarbazide and evaluated *in vitro* for antimicrobial activity against *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphyllococcus aureus.*

Methods: 4-(3-Chlorophenyl)-3-thiosemicarbazones were synthesized and allowed to react with 2,4′-dibromoacetophenone to give 2-hydrazono-2(3*H*)-thiazoles in excellent yields. The synthesized compounds were characterized by spectroscopic methods as well as elemental analyses. They were screened for their antimicrobial activity by using the agar diffusion method.

Results: The antimicrobial data suggested that all compounds showed significant activity against *E. coli* and *C. albicans* compared to the standard drug. Only 7b and 7d exhibited moderate activity against *P. aeruginosa*.

Conclusion: Compounds bearing a substituted phenyl or indolyl group at the hydrazone moiety have considerable antimicrobial activity than those with a cycloalkyl group.

Keywords: 2,3-Dihydrothiazole, 4-(3-Chlorophenyl)-3-thiosemicarbazide, Antimicrobial activity

INTRODUCTION

The synthesis of thiazole derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural and synthetic products contain such heterocyclic nucleus, such as vitamin B_1 (thiamin), sulfathiazole, epothilone, ritonavir, abafungin and tiazofurin [1, 2]. Different thiazole bearing compounds are known to possess many biological activities such as anti-inflammatory [3], antibacterial [4], antifungal [5], herbicidal and insecticidal activity [6]. Moreover, 2-hydrazonothiazoles have also attracted increasing attention due to their numerous biological activities against *E. coli*, *P. aeruginosa*, *Aspergillus flavus*, *S. aureus*, *B. subtilis*, *Penicillium marneffei*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Aspergillus niger*, *C. albicans*, *Cryptococcus neoformans* [7-10], *Mycobacterium tuberculosis* [11], *plasmodium falciparum* [12], *Trypanosoma cruzi* [13], recombinant histone acetyltransferases [14], human recombinant monamine oxidase B [15] and carcinoma cell lines [16]. 2-[(2,4-Diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazono]-4-phenyl-2,3-dihydrothiazoles were also found to show antibacterial and antifungal activities against some selected microorganisms [17]. In continuation of our work on 2-thiazolylhydrazones [18], we report herein the synthesis, characterization and antimicrobial activity of some 2-hydrazono-2(3*H*)-thiazole derivatives.

MATERIALS AND METHODS

Starting chemicals and reagents used in this study were purchased from Sigma-Aldrich. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Mass spectra were recorded using an Electron Impact (EI) mode on a Shimadzu Qp-2010 GCMS apparatus operating at an electron ionizing energy of 70 eV. TLC was performed on 0.25 mm aluminum silica gel plates (60 F_{254}, Fluka) using ethyl acetate: petroleum ether (2:3) and ethyl acetate: methylene chloride (4:1) as eluents; the spots were detected by using UV light absorption. FT-IR spectra were measured on a Bruker Tensor 37 FT-IR spectrometer. ^{1}H NMR spectra were recorded on a JEOL JNM ECA-500 MHz spectrometer at 500 MHz spectrometer. The chemical shifts are reported in δ units and coupling constants (*J*) are given in hertz. Microanalyses were performed on an elementar vario EL III element analyzer in Cairo University and elementar vario MICRO cube in Advanced Technology and New Materials Research Institute Laboratories, City of Scientific Research and Technological Applications, Alexandria, Egypt. Antimicrobial tests were measured at the Pharmaceutical Microbiology Department, Faculty of Pharmacy, Alexandria University.

Synthesis of 4-(3-chlorophenyl)-3-thiosemicarbazide (2)

To a solution of 3-chloroaniline (1) (31.89 g, 0.25 mol) in ethanol (50 ml) was slowly added 40 ml of concentrated ammonium hydroxide (sp. gr. 0.91). The reaction mixture was cooled below 30 °C and carbon disulphide (15 ml) was added dropwise during a period of 15 min. After one hour, an aqueous solution of sodium salt of monochloroacetic acid (0.25 mol) was added to the reaction mixture and this was followed by the addition of hydrazine hydrate (0.25 mol, 80%). The mixture was cooled overnight in a refrigerator and the crude thiosemicarbazide which separated out was filtered and recrystallized from ethanol. White crystals, 84% yield, m. p.: 118-120 °C; IR (KBr) ν_{max} (cm^{-1}): 3331.96, 3279.59, 3195.80, 1630.62, 1590.72, 1522.94, 1487.31, 1253.81, 1210.92, 1066.11, 966.14; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 4.85 (brs, 2H, D_2O-exchang., NH_2), 6.94 (d, *J*=6.9Hz, 1H, Ar-H), 7.26 (t, *J*=7.65Hz, 1H, Ar-H), 7.53 (d, *J*=6.9Hz, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 9.26 (s, 2H, D_2O-exchang., NH_2).

Synthesis of 4-(3-chlorophenyl)-3-thiosemicarbazones (3a-e, 8 and 11): General procedure

4-(3-Chlorophenyl)-3-thiosemicarbazide (2) (2.01 g, 10 mmol) in absolute ethanol (30 ml) and glacial acetic acid (1.0 ml) was warmed on a steam bath to a clear solution and added to an aldehyde or ketone (10 mmol) in absolute ethanol (20 ml). The reaction mixture was heated under reflux for 1 h and the product that separated out on cooling was filtered, washed with a little ethanol and dried. It was recrystallized from ethanol to give the title compounds.

1-Phenylmethylidene-4-(3-chlorophenyl)-3-thiosemicarbazide (3a)

White plates, 78% yield, m. p.: 144-146 °C; IR (KBr) ν_{max} (cm^{-1}): 3325.62, 3294.44, 3161.46, 2935.39, 1587.25, 1538.12, 1424.18, 1258.66, 1198.15; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 7.22, 7.23 (dd, *J*=7.6, 1.5Hz, 1H, Ar-H), 7.36 (t, *J*=8.4Hz, 1H, Ar-H), 7.39-7.41 (m, 3H, Ar-H), 7.56 (d, *J*=8.4Hz, 1H, Ar-H), 7.72 (t, *J*=1.5Hz, 1H, Ar-H), 7.86, 7.87 (dd, *J*=7.6, 2.3Hz, 2H, Ar-H), 8.13 (s, 1H, CH=N), 10.15 (s, 1H, D_2O-exchang., NH-Ar), 11.93 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 291.05 [16.16%] [M^++2], 289.05 [43.64%] [M^+]; Anal. Calcd for $C_{14}H_{12}ClN_3S$: C, 58.03; H, 4.17; N, 14.50. Found: C, 58.30; H, 4.46; N, 14.51%.

1-(4-Nitrophenylmethylidene)-4-(3-chlorophenyl)-3-thiosemicarbazide (3b)

Yellow crystals, 86% yield, m. p.: 228-230 °C; IR (KBr) ν_{max} (cm^{-1}): 3340.99, 3307.06, 3134.14, 2982.97, 1587.62, 1544.32, 1429.19, 1337.55, 1251.60, 1187.19; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 7.25, 7.26 (dd, *J*=8.4, 1.55Hz, 1H, Ar-H), 7.38 (t, *J*=8.4Hz, 1H, Ar-H), 7.53 (d, *J*=8.4Hz, 1H, Ar-H), 7.68 (t, *J*=1.55Hz, 1H, Ar-H), 8.15 (d, *J*=9.15Hz, 2H, Ar-H), 8.21 (s, 1H, CH=N), 8.23 (d, *J*=9.15Hz, 2H, Ar-H), 10.34 (s, 1H, D_2O-exchang., NH-Ar), 12.19 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 336.05 [6.08%] [M^++2], 334.00 [20.89%] [M^+]; Anal. Calcd for $C_{14}H_{11}ClN_4O_2S$: C, 50.23; H, 3.31; N, 16.74. Found: C, 50.53; H, 3.12; N, 16.83%.

1-(4-Methoxyphenylmethylidene)-4-(3-chlorophenyl)-3-thiosemicarbazide (3c)

Pale yellow plates, 80% yield, m. p.: 166-168 °C; IR (KBr) ν_{max} (cm^{-1}): 3325.19, 3148.98, 3016.60, 2923.61, 1605.01, 1546.05, 1417.96, 1248.59, 1199.54, 1164.33, 1070.84; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 3.77 (s, 3H, OCH_3), 6.94 (d, *J*=9.15Hz, 2H, Ar-H), 7.21, 7.22 (dd, *J*=9.15, 1.55Hz, 1H, Ar-H), 7.35 (t, *J*=8.4Hz, 1H, Ar-H), 7.56 (d, *J*=8.4Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.81 (d, *J*=8.4Hz, 2H, Ar-H), 8.08 (s, 1H, CH=N), 10.08 (s, 1H, D_2O-exchang., NH-Ar), 11.82 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 321.05 [8.09%] [M^++2], 319.05 [24.94%] [M^+]; Anal. Calcd for $C_{15}H_{14}ClN_3OS$: C, 56.33; H, 4.41; N, 13.14. Found: C, 56.57; H, 4.36; N, 13.01%.

1-(2-Chlorophenylmethylidene)-4-(3-chlorophenyl)-3-thiosemicarbazide (3d)

Yellow crystals, 83% yield, m. p.: 192-194 °C; IR (KBr) ν_{max} (cm^{-1}): 3300.58, 3222.80, 3097.35, 2990.48, 1589.97, 1541.66, 1420.48, 1251.66, 1186.07; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 7.23 (d, *J*=7.65Hz, 1H, Ar-H), 7.35-7.41 (m, 3H, Ar-H), 7.46 (d, *J*=7.65Hz, 1H, Ar-H), 7.54 (d, *J*=8.4Hz, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 8.41 (d, *J*=6.9Hz, 1H, Ar-H), 8.57 (s, 1H, CH=N), 10.23 (s, 1H, D_2O-exchang., NH-Ar), 12.10 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 326.95 [5.38%] [M^++4], 325.00 [23.09%] [M^++2], 323.00 [32.08%] [M^+]; Anal. Calcd for $C_{14}H_{11}Cl_2N_3S$: C, 51.86; H, 3.42; N, 12.96. Found: C, 52.00; H, 3.56; N, 13.07%.

(*E/Z*)-1-(2-Hydroxyphenylmethylidene)-4-(3-chlorophenyl)-3-thiosemicarbazide (3e)

White plates, 79% yield, m. p.: 176-178 °C; IR (KBr) ν_{max} (cm^{-1}): 3386.61, 3140.91, 2987.50, 1616.91, 1591.62, 1543.97, 1426.31, 1274.06, 1210.14, 1154.97, 1082.55; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): [6.81 (t, *J*=6.85Hz, Ar-H), 6.84 (d, *J*=7.65Hz, Ar-H), 6.92-6.95 (m, Ar-H), 7.19-7.22 (m, Ar-H), 7.33-7.36 (m, Ar-H), 7.55 (d, *J*=8.4Hz, Ar-H), 7.65, 7.67 (dd, *J*=7.65, 1.5Hz, Ar-H), 7.73 (t, *J*=1.5Hz, Ar-H), 8.05 (d, *J*=6.9Hz, Ar-H), the total integral of Ar-H=8H], 8.46 (s, 0.71H, CH=N), 8.97 (s, 0.29H, CH=N), 9.98 (s, 0.71H, D_2O-exchang., OH), 11.10 (s, 0.29H, D_2O-exchang., OH), 10.08 (s, 1H, D_2O-exchang., NH-Ar), 11.86 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 307.00 [19.12%] [M^++2], 305.00 [52.79%] [M^+].

1-Cyclopentylidene-4-(3-chlorophenyl)-3-thiosemicarbazide (8)

White crystals, 76% yield, m. p.: 152-154 °C; IR (KBr) ν_{max} (cm^{-1}): 3311.45, 3172.21, 2953.46, 2878.85, 1586.87, 1518.46, 1416.31, 1272.07, 1193.94; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 1.66 (quintet, *J*=6.85Hz, 2H, -CH_2-*CH_2*-CH_2CH_2-), 1.74 (quintet, *J*=6.85Hz, 2H,-CH_2CH_2-*CH_2*-CH_2-), 2.37 (t, *J*=7.65Hz, 2H, -*CH_2*-$CH_2CH_2CH_2$-), 2.41 (t, *J*=7.65Hz, 2H, -CH_2CH_2-CH_2-*CH_2*-), 7.15, 7.16 (dd, *J*=8.4, 2.3Hz, 1H, Ar-H), 7.30 (t, *J*=8.4Hz, 1H, Ar-H), 7.51 (d, *J*=7.65Hz, 1H, Ar-H), 7.79 (t, *J*=2.3Hz, 1H, Ar-H), 9.89 (s, 1H, D_2O-exchang., NH-Ar), 10.36 (s, 1H, D_2O-exchang., NH-CS); MS (*m/z*): 269.05 [8.54%] [M^++2], 267.05 [22.75%] [M^+]; Anal. Calcd for $C_{12}H_{14}ClN_3S$: C, 53.82; H, 5.27; N, 15.69. Found: C, 53.75; H, 5.01; N, 15.60%.

1*H*-Indole-2, 3-dione-3-*N*-(3'-chlorophenyl)thiosemicarbazone (11)

Yellow solid, 87% yield, m. p.: 234-236 °C; IR (KBr) ν_{max} (cm^{-1}): 3314.61, 3184.97, 1691.71, 1620.79, 1588.24, 1528.23, 1464.48, 1426.49, 1168.81; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 6.90 (d, *J*=7.65Hz, 1H, Ar-H), 7.08 (t, *J*=6.85Hz, 1H, Ar-H), 7.29, 7.30 (dd, *J*=8.4, 1.55Hz, 1H, Ar-H), 7.34 (t, *J*=7.65Hz, 1H, Ar-H), 7.41 (t, *J*=8.4Hz, 1H, Ar-H), 7.59, 7.61 (dd, *J*=9.15, 1.5Hz, 1H, Ar-H), 7.74 (d, *J*=7.65Hz, 1H, Ar-H), 7.75 (t, *J*=2.3Hz, 1H, Ar-H), 10.84 (s, 1H, D_2O-exchang., NH), 11.25 (s, 1H, D_2O-exchang., NH), 12.82 (s, 1H, D_2O-exchang., NH); MS (*m/z*): 331.95 [10.54%] [M^++2], 330.00 [27.74%] [M^+]; Anal. Calcd for $C_{15}H_{11}ClN_4OS$: C, 54.46; H, 3.35; N, 16.94. Found: C, 54.64; H, 3.36; N, 16.91%.

Synthesis of 2-hydrazono-2,3-dihydrothiazoles (7a-e, 9 and 12): General procedure

A solution of 3a-e, 8 or 11 (10 mmol) in *iso*-propyl alcohol (25 ml) and *N,N*-dimethylformamide (2.0 ml) was treated with the solution of 4 (2.78 g, 10 mmol) in *iso*-propyl alcohol (25 ml) and the reaction mixture was heated under reflux for 2 h and left to cool to room temperature, the reaction progress was monitored by TLC using ethyl acetate: methylene chloride (4:1 *v/v*). The separated product was filtered, washed twice with saturated solution of $NaHCO_3$ (30 ml) and recrystallized from a mixture of ethanol and *N,N*-dimethylformamide.

4-(4-Bromophenyl)-3-(3-chlorophenyl)-2-(phenylmethylidenehydrazono)-2(3*H*)-thiazole (7a)

Yellow crystals, 81% yield, m. p.: 232-234 °C; IR (KBr) ν_{max} (cm^{-1}): 3116.39, 3055.36, 2922.82, 1602.15, 1578.66, 1340.79; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 6.69 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.11 (d, *J*=8.4Hz, 2H, Ar-H), 7.16, 7.17 (tt, *J*=8.4, 2.3Hz, 1H, Ar-H), 7.32-7.39 (m, 5H, Ar-H), 7.44 (d, *J*=8.4Hz, 2H, Ar-H), 7.51 (s, 1H, Ar-H), 7.65 (d, *J*=6.9Hz, 2H, Ar-H), 8.17 (s, 1H, CH=N); MS (*m/z*): 470.95 [30.98%] [M^++4], 468.95 [100.00%] [M^++2], 467.00 [72.58%] [M^+]; Anal. Calcd for $C_{22}H_{15}BrClN_3S$: C, 56.36; H, 3.23; N, 8.96. Found: C, 56.63; H, 3.22; N, 8.76%.

4-(4-Bromophenyl)-3-(3-chlorophenyl)-2-(4-nitrophenylmethylidenehydrazono)-2(3*H*)-thiazole (7b)

Orange crystals, 85% yield, m. p.: 240-242 °C; IR (KBr) ν_{max} (cm^{-1}): 3108.93, 1588.62, 1497.52, 1335.23; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 6.79 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.12 (d, *J*=8.4Hz, 2H, Ar-H), 7.19, 7.20 (dd, *J*=6.85, 1.5Hz, 1H, Ar-H), 7.35-7.40 (m, 2H, Ar-H), 7.46 (d, *J*=8.4Hz, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 7.87 (d, *J*=9.15Hz, 2H, Ar-H), 8.22 (d, *J*=9.15Hz, 2H, Ar-H), 8.28 (s, 1H, CH=N); MS (*m/z*): 515.95 [29.92%] [M^++4], 514.00 [100.00%] [M^++2], 512.00 [73.29%] [M^+]; Anal. Calcd for $C_{22}H_{14}BrClN_4O_2S$: C, 51.43; H, 2.75; N, 10.90. Found: C, 51.35; H, 3.16; N, 10.73%.

4-(4-Bromophenyl)-3-(3-chlorophenyl)-2-(4-methoxyphenylmethylidenehydrazono)-2(3*H*)-thiazole (7c)

Pale yellow crystals, 82% yield, m. p.: 218-220 °C; IR (KBr) ν_{max} (cm^{-1}): 3113.08, 3062.84, 2998.49, 2963.23, 1604.30, 1521.50, 1341.76, 1250.99, 1162.84, 1070.28; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 3.75 (s, 3H, OCH_3), 6.65 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 6.93 (d, *J*=8.4Hz, 2H, Ar-H), 7.10 (d, *J*=8.4Hz, 2H, Ar-H), 7.14, 7.15 (dd, *J*=6.5, 1.5Hz, 1H, Ar-H), 7.32-7.35 (m, 2H, Ar-H), 7.43 (d, *J*=8.4Hz, 2H, Ar-H), 7.48 (s, 1H, Ar-H), 7.59 (d, *J*=8.4Hz, 2H, Ar-H), 8.10 (s, 1H, CH=N); MS (*m/z*): 501.00 [32.16%] [M^++4], 499.00 [100.00%] [M^++2], 497.00 [75.17%] [M^+]; Anal. Calcd for $C_{23}H_{17}BrClN_3OS$: C, 55.38; H, 3.44; N, 8.42. Found: C, 55.22; H, 3.68; N, 8.11%.

4-(4-Bromophenyl)-2-(2-chlorophenylmethylidenehydrazono)-3-(3-chlorophenyl)-2(3*H*)-thiazole (7d)

Yellow crystals, 84% yield, m. p.: 246-248 °C; IR (KBr) ν_{max} (cm^{-1}): 3112.46, 3062.72, 1595.28, 1557.64, 1340.03; ^{1}H NMR (500 MHz,

DMSO-d_6) (δppm): 6.73 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.10 (d, *J*=8.4Hz, 2H, Ar-H), 7.20 (d, *J*=6.9Hz, 1H, Ar-H), 7.37-7.38 (m, 4H, Ar-H), 7.44-7.50 (m, 4H, Ar-H), 7.94 (s, 1H, Ar-H), 8.33 (s, 1H, CH=N); MS (*m/z*): 506.90 [5.06%] [M^++6], 504.90 [27.16%] [M^++4], 502.90 [53.75%] [M^++2], 500.90 [31.24%] [M^+]; Anal. Calcd for $C_{22}H_{14}BrCl_2N_3S$: C, 52.51; H, 2.80; N, 8.35. Found: C, 52.49; H, 2.49; N, 8.04%.

4-(4-Bromophenyl)-3-(3-chlorophenyl)-2-(2-hydroxyphenyl-methylidenehydrazono)-2(3*H*)-thiazole (7e)

Pale yellow crystals, 80% yield, m. p.: 224-226 °C; IR (KBr) ν_{max} (cm^{-1}): 3440.24, 3108.74, 1604.74, 1577.05, 1521.39, 1340.11, 1266.92, 1073.89; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 6.73 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 6.86 (t, *J*=6.85Hz, 1H, Ar-H), 6.88 (d, *J*=8.4Hz, 1H, Ar-H), 7.12 (d, *J*=8.4Hz, 2H, Ar-H), 7.19-7.23 (m, 2H, Ar-H), 7.34-7.37 (m, 2H, Ar-H), 7.41, 7.43 (dd, *J*=8.4, 2.3Hz, 1H, Ar-H), 7.44 (d, *J*=8.4Hz, 2H, Ar-H), 7.54 (t, *J*=2.3Hz, 1H, Ar-H), 8.41 (s, 1H, CH=N), 11.02 (brs, 1H, OH); MS (*m/z*): 486.90 [30.97%] [M^++4], 484.90 [100.00%] [M^++2], 482.95 [74.68%] [M^+]; Anal. Calcd for $C_{22}H_{15}BrClN_3OS$: C, 54.50; H, 3.12; N, 8.67. Found: C, 54.17; H, 3.14; N, 8.40%.

4-(4-Bromophenyl)-3-(3-chlorophenyl)-2-(cyclopentylidene-hydrazono)-2(3*H*)-thiazole (9)

Pale yellow crystals, 78% yield, m. p.: 180-182 °C; IR (KBr) ν_{max} (cm^{-1}): 3113.46, 3072.44, 2959.95, 2874.58, 1637.92, 1587.17, 1550.95, 1349.43; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 1.57-1.62 (m, 4H,-CH_2-*CH_2*-*CH_2*-CH_2-), 2.17 (t, *J*=6.9Hz, 2H, -*CH_2*-CH_2-CH_2-CH_2-), 2.29 (t, *J*=6.9Hz, 2H, -CH_2-CH_2-CH_2-*CH_2*-), 6.56 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.04-7.05 (m, 1H, Ar-H), 7.07 (d, *J*=8.4Hz, 2H, Ar-H), 7.29 (d, *J*=6.1Hz, 2H, Ar-H), 7.41 (s, 1H, Ar-H), 7.42 (d, *J*=8.4Hz, 2H, Ar-H); MS (*m/z*): 448.95 [30.60%] [M^++4], 446.95 [100.00%] [M^++2], 444.95 [74.64%] [M^+]; Anal. Calcd for $C_{20}H_{17}BrClN_3S$: C, 53.76; H, 3.84; N, 9.40. Found: C, 53.55; H, 3.87; N, 9.13%.

3-[4-(4-Bromophenyl)-3-(3-chlorophenyl)-thiazol-2(3*H*)-ylide-nehydrazono]indolin-2-one (12)

Orange plates, 82% yield, m. p.: 262-264 °C; IR (KBr) ν_{max} (cm^{-1}): 3171.98, 3102.93, 3067.59, 1703.10, 1614.76, 1588.96, 1333.26; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 6.71 (t, *J*=6.85Hz, 1H, Ar-H), 6.75 (d, *J*=7.65Hz, 1H, Ar-H), 7.02 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.15 (t, *J*=7.65Hz, 1H, Ar-H), 7.19 (d, *J*=7.65 Hz, 3H, Ar-H), 7.41 (t, *J*=7.65Hz, 1H, Ar-H), 7.49 (d, *J*=8.4Hz, 4H, Ar-H), 7.78 (t, *J*=2.3Hz, 1H, Ar-H), 10.49 (s, 1H, NH); MS (*m/z*): 511.85 [29.48%] [M^++4], 509.90 [100.00%] [M^++2], 507.90 [70.02%] [M^+]; Anal. Calcd for $C_{23}H_{14}BrClN_4OS$: C, 54.19; H, 2.77; N, 10.99. Found: C, 54.09; H, 2.80; N, 11.24%.

Synthesis of 1-acetyl-3-[(4-(4-bromophenyl)-3-(3-chlorophenyl) -thiazol-2(3*H*)-ylidenehydrazono]indolin-2-one (13)

A cold suspension of compound 12 (0.76 g, 1.5 mmol) in dry pyridine (10 ml) was treated with acetic anhydride (10 ml) and the mixture was kept for 24 h at room temperature. It was poured onto crushed ice and the product was filtered off, repeatedly washed with water, dried and recrystallized from ethanol as red crystals. 78% Yield, m. p.: 292-294 °C; IR (KBr) ν_{max} (cm^{-1}): 3102.66, 3070.88, 1744.12, 1711.19, 1590.42, 1337.51; ^{1}H NMR (500 MHz, DMSO-d_6) (δppm): 2.56 (s, 3H, *N*-$COCH_3$), 6.96 (t, *J*=7.65Hz, 1H, Ar-H), 7.13 (s, 1H, C_5-H of 2(3*H*)-thiazole ring), 7.19 (d, *J*=8.4Hz, 2H, Ar-H), 7.24 (d, *J*=8.4Hz, 1H, Ar-H), 7.29 (t, *J*=7.65Hz, 1H, Ar-H), 7.45 (t, *J*=8.4Hz, 1H, Ar-H), 7.50 (d, *J*=7.65Hz, 3H, Ar-H), 7.71 (d, *J*=7.65Hz, 1H, Ar-H), 7.78 (t, *J*=2.3Hz, 1H, Ar-H), 8.09 (d, *J*=7.65Hz, 1H, Ar-H); MS (*m/z*): 553.90 [31.03%] [M^++4], 551.95 [100.00%] [M^++2], 549.95 [71.30%] [M^+]; Anal. Calcd for $C_{25}H_{16}BrClN_4O_2S$: C, 54.41; H, 2.92; N, 10.15. Found: C, 54.62; H, 3.01; N, 10.16%.

Antibacterial and antifungal testing

The synthesized 2-hydrazono-2,3-dihydrothiazoles have been screened for *in vitro* antibacterial activity against Gram-positive bacteria *B. subtilis* (ATCC 19659), *S. aureus* (ATCC 6538P) and Gram-negative bacteria *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027) and antifungal activity against *C. albicans* (ATCC 2091) by an agar diffusion technique [19], using a 1 mg/1 ml solution in DMSO. Each tested organism was cultured in 3 ml of sterile nutrient broth and incubated for 18 h at 37 °C. Aseptically 0.4 ml was taken by a glass pipette from resultant microbial growth into 40 ml of warm agar in one sterile flask for each organism. The seeded agar was poured into sterile Petri dishes (~15 cm in diameter) onto a level surface to obtain a layer of about 4 mm thickness and the plates were then left to solidify. Cups of 8 mm in diameter were made by a cork borer, the sample size for all the compounds was fixed at 65 µl and one cup filled by DMSO as a control. The plates were then incubated at 37 °C for 24 h. The diameter of each resultant growth inhibition zone (IZ in mm) was accurately measured in three different directions, and its mean was calculated. Clotrimazole and ciprofloxacin were used as standard drugs.

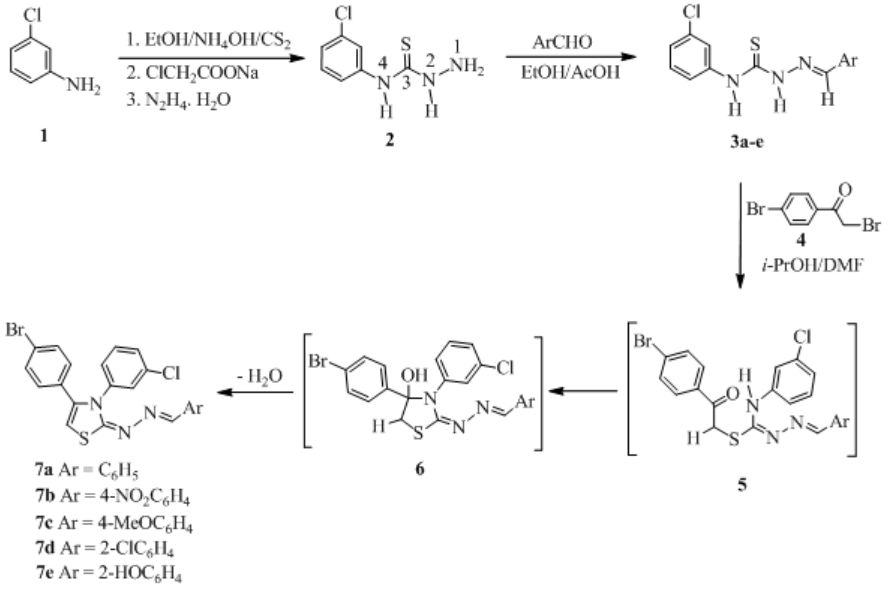
RESULTS AND DISCUSSION

In this work, 4-(3-chlorophenyl)-3-thiosemicarbazide (2) was prepared in one-pot in 84% yield *via* a multicomponent coupling reaction of 3-chloroaniline (1), aqueous ammonia, carbon disulfide, sodium 2-chloroacetate and hydrazine hydrate in ethanol at room temperature (scheme 1). A series of 1-arylmethylidene-4-(3-chlorophenyl)-3-thiosemicarbazides (3a-e) were synthesized in 78-86% yields by condensing 2 with benzaldehyde, 4-nitro-benzaldehyde, 4-methoxybenzaldehyde, 2-chlorobenzaldehyde and 2-hydroxybenzaldehyde in absolute ethanol in the presence of a catalytic amount of glacial acetic acid. Their FT-IR spectra showed absorption bands at 3140.91-3386.61 cm^{-1}characteristic for the NH stretching vibrations and 1538.12-1616.91 cm^{-1} due to the C=C and C=N groups. They also showed absorption bands in 1417.96-1429.19 cm^{-1} and 1186.07-1274.06 cm^{-1} due to N-CS-N and C=S stretching vibrations, respectively. No SH bands were found in these compounds in the region of 2500-2600 cm^{-1}, the range in which the SH-stretching vibrations are most likely to appear. Thus clearly shows that there is no thiol-thione tautomerism of these compounds in the solid state. The ^{1}H NMR spectra of thiosemicarbazones (3a-d) showed a sharp singlet at δ 8.08-8.57 ppm indicating the presence of azomethine proton (CH=N), whereas the NH-Ar and NH-CS protons appeared as two singlet peaks at δ 10.08-10.34 and 11.82-12.19 ppm, respectively [20].

The presence of *ortho* hydroxyl group with respect to the azomethine group of 1-(2-hydroxyphenylmethylidene)-4-(3-chlorophenyl)-3-thiosemicarbazide (3e) has been regarded as one of the important elements favoring the existence of intramolecular hydrogen bond and also the tautomerism accounting for the formation of either phenol-imine (HO…N) or keto-amine (O…. HN) tautomers (fig. 1). Such observations have been investigated in the solid state and in solution by using both IR and ^{1}H NMR spectroscopic methods [21-23]. The IR spectrum of 3e showed a relatively strong band at 1616.91 cm^{-1} due to CH=N stretching as in Schiff bases having intramolecular hydrogen bonds between the nitrogen atom of azomethine group and *ortho* hydroxyl group [24-26]. Furthermore, the IR spectrum of 3e exhibited neither SH nor keto-amine C=O stretching frequencies, indicating the absence of thiol and keto-amine tautomers. According to ^{1}H NMR spectrum an equilibrium mixture of two geometrical isomers (*E* and *Z*) have been observed in DMSO-d_6, whereby the OH signal of *E*-isomer was broad and was found at δ 9.98 ppm confirming the existence of the phenol-imine (HO…. N) tautomer, while the OH signal of *Z*-isomer appeared as a sharp singlet at δ 11.10 ppm. The azomethine (CH=N) proton of the equilibrium mixture appeared as two singlet peaks at δ 8.46 and 8.97 ppm in a ratio of 2.7:1, accounting for the fact that the *E*-isomer was more favored and stable than the *Z*-configuration in a solution of DMSO. Moreover, no signals were observed for the HN-**CH** and **HN**-CH protons suggesting the absence of keto-amine (O…. HN) tautomer.

Cyclocondensation of compounds 3a-e with 2,4′-dibromo-acetophenone (4) was carried out in refluxing *iso* propyl alcohol and little *N,N*-dimethylformamide to give 2-arylidenehydrazono-4-(4-bromophenyl)-3-(3-chlorophenyl)-2(3*H*)-thiazoles (7a-e) as showed in scheme 2. The reaction was completed in 2 h and the yields of products ranged in 80-85%. The choice of our solvent let the reactants completely dissolved and the obtained reaction products precipitated upon cooling. The absence of any bands for the NH groups in their IR spectra and the presence of absorption bands at

1335.23-1341.76 cm^{-1} due to N-C-S bending vibrations provided confirmatory evidence for ring closure. The ^1H NMR spectra of 7a-e showed a singlet at δ 6.65-6.79 ppm due to C_5-H of the 2,3-dihydrothiazole ring and a singlet at δ 8.10-8.41 ppm corresponding to the azomethine proton. A suitable mechanism for the formation of 7 may involve initial nucleophilic attack of thiol sulfur atom of 3 to the *sp3* carbon of 4 forming the *S*-phenacylacted intermediate 5, which underwent a nucleophilic attack by the lone pair of electrons of the nitrogen atom to the carbonyl carbon atom giving 6. Subsequent elimination of a water molecule afforded 7 [27, 28].



Scheme 1: Synthesis of 2-hydrazono-2,3dihydrathiazole derivatives 7a-e

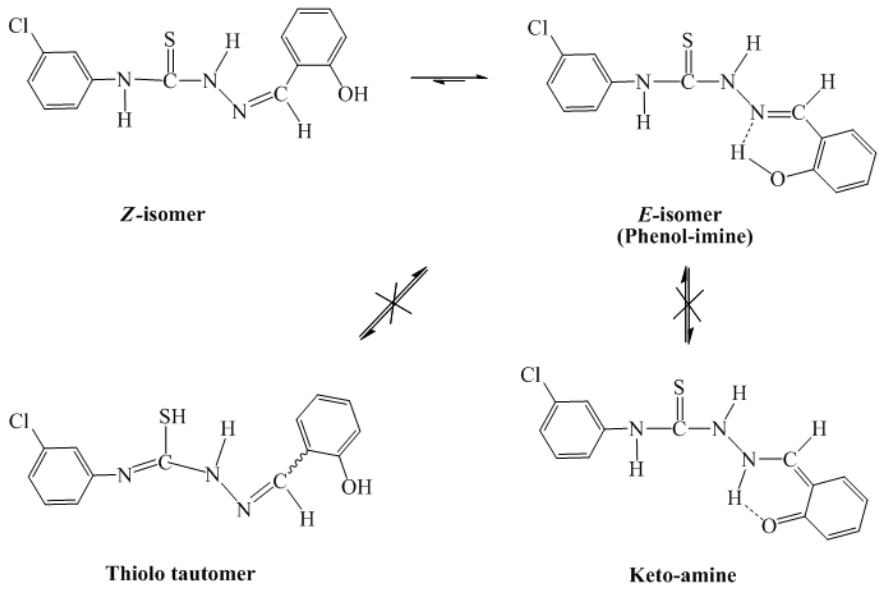


Fig. 1: Tautomerism and intramolecular hydrogen bonding of 3e

Condensation of 2 with cyclopentanone afforded thiosemicarbazone 8, its cyclocondensation with 4 gave thiazole 9 (scheme 2). In its ^1H NMR spectrum, the protons of the cyclopentyl ring residue appeared as two quintets at δ 1.66, 1.74 ppm (*J*=6.85Hz) and two triplets at δ 2.37, 2.41 ppm (*J*=7.65Hz) indicating that cyclopentyl ring may exist in solution in a puckered conformation.

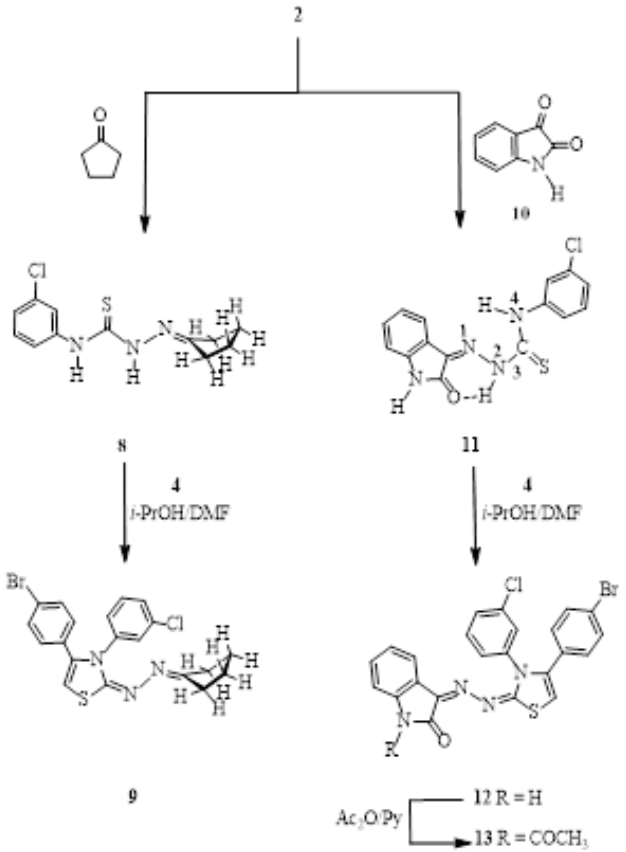
Indole-2,3-dione-3-*N*-(3'-chlorophenyl) thiosemicarbazone (11) was obtained in 87% yield by refluxing of indole-2, 3-dione (10) with 2 in ethanol and a few drops of glacial acetic acid for 1 h. The presence of a low field N-H proton at *δ* 12.82 ppm in its ^1H NMR spectrum indicated its existence in *Z*-configuration as a result of intramolecular hydrogen bonding between the ketonic oxygen at C-2 of indoline ring and hydrazine hydrogen at *N*-2 of hydrazone moiety [29]. It was cyclized with 4 to give thiazole 12 in good yield.

Acetylation of compound 12 with acetic anhydride in pyridine was carried out at room temperature to give the corresponding *N*-acetyl derivative 13, whose IR spectrum showed bands at 1744.12 and 1711.19 cm^{-1} due to C=O stretching vibrations of the indole ring and *N*-$COCH_3$ group, respectively. The latter was also confirmed by the presence of a sharp singlet at δ 2.56 ppm corresponding to its methyl protons in the ^1H NMR spectrum. The protons at C_5 of the 2, 3-dihydrothiazole ring of 12 and 13 were observed as a singlet at δ 7.02 and 7.13 ppm, respectively.

Antimicrobial activity

The preliminary antimicrobial results of the synthesized thiazoles are listed in table 1. The results revealed that these compounds exhibited varying degrees of inhibition against the fungus *C. albicans* (ATCC 2091), Gram-negative bacteria *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 9027) and Gram-positive bacteria *B. subtilis* (ATCC 19659) and *S. aureus* (ATCC 6538P). The hydrazones 7a-e, 12 and 13 showed good growth inhibition activity against *C. albicans* (IZ = 20-23 mm) and *E. coli* (IZ = 20-21 mm).

Alternatively, only 7b and 7d showed considerable activity against *P. aeruginosa*. In regard to structure, the most active compound against *C. albicans* was also 7d (IZ = 23 mm), carrying 4-chlorophenyl group on the hydrazone moiety. The indolines 12 and 13 showed equal activity (IZ = 20 mm) to that of 7a-c, while 9 having cyclopentyl group at the hydrazone part possesses activity to a lower extent (IZ = 17 mm). By contrast, no substantial difference of activity was found for the hydrazones 7a-e, 12 and 13 against *E. coli* (IZ = 20-21 mm), while 9 exhibited lower activity (IZ = 16 mm). All the tested compounds were found to be inactive against *B. subtilis* and *S. aureus*. Comparison of inhibitory activity of the synthesized compounds towards *C. albicans* with the previously reported 2-hydrazonothiazoles [18] is listed in table 2. The results showed that compounds 7a-d possessed strong inhibitory activity against *C. albicans* compared to the corresponding 2-hydrazonothiazole derivatives 14a-d. This observation suggested that the combination of the 2,3-dihydrothiazole ring with the 3-chlorophenyl substituent at *N*-3 of 7a-d enhanced their activity against the tested microorganism. Moreover, compounds 12 and 13 with indolyl group at the hydrazone moiety exhibited promising activity compared to the reference compounds 14a-d. However, the cyclopentyl derivative 9 showed activity higher than 14a-d but to a lower extent than 7a-d, 12 and 13.



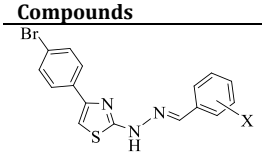
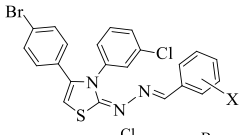
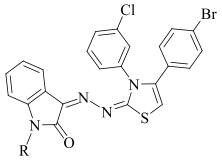
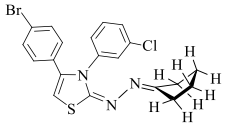
Scheme 2: Synthesis of 2-hydrazono-2,3-dihydrathiazole with cycloalkyl and indolyl groups

Table 1: The mean* growth inhibition zones (IZ in mm) and % inhibition of 2-hydrazono-2,3-dihydrothiazoles**

	C. albicans		***E. coli***		***P. aeruginosa***		***B. subtilis***	***S. aureus***
Compd.	IZ	% inhibition	IZ	% inhibition	IZ	% inhibition	IZ	IZ
7a	20	41.17	21	23.33	9	---	9	9
7b	20	41.17	20	20.00	12	10.00	9	9
7c	20	41.17	20	20.00	9	---	9	9
7d	23	58.82	21	23.33	16	23.33	9	9
7e	20	41.17	21	23.33	9	---	9	9
9	17	23.52	16	6.66	9	---	9	9
12	20	41.17	20	20.00	9	---	9	9
13	20	41.17	20	20.00	9	---	9	9
DMSO	13	76.47	14	46.66	9	30.00	9	9
Clotrimazole	17	---	---	---	---	---	---	---
Ciprofloxacin	---	---	30	---	30	---	30	30

*The diameter of each resultant growth inhibition zone is accurately measured in three different directions (n = 3) and its mean was calculated; Errors are within ±5%; ** % Inhibition = [(IZ of tested compound–IZ of DMSO)/ IZ of reference drug] × 100

Table 2: Comparison of activity towards *C. albicans* with the previously reported 2-hydrazonothiazoles

Compounds	Inhibition zone (IZ)	% Inhibition	
	14a X = H	13 [18]	2.50
	14b X = 4-NO_2	12 [18]	0.00
	14c X = 4-OCH_3	12 [18]	0.00
	14d X = 2-Cl	12 [18]	0.00
	7a X = H	20	41.17
	7b X = 4-NO_2	20	41.17
	7c X = 4-OCH_3	20	41.17
	7d X = 2-Cl	23	58.82
	12 R = H	20	41.17
	13 R = $COCH_3$	20	41.17
	9	17	23.52

CONCLUSION

Several substituted 2-hydrazono-2(3*H*)-thiazole derivatives were synthesized by reacting of 4-(3-chlorophenyl)-3-thiosemicarbazide with different aldehydes and ketones, followed by cyclization with 2, 4′-dibromoacetophenone in a second step. They were tested for antimicrobial activity against *E. coli*, *C. albicans*, *P. aeruginosa*, *B. subtilis* and *S. aureus* and showed good activity against *E. coli* and *C. albicans*.

CONFLICT OF INTERESTS

Declared none

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