INTRODUCTION

Pyrazoline derivatives are associated with broad spectrum of biological activities. Derivatives of 3,5-diaryl-4,5-dihydropyrazole were reported to exhibit antiinflammatory, analgesic1,2, antidepressant3-5, hypotensive6, trypanocidal7 and antitumor8,9 activities. Numerous studies have been published on the antimicrobial activity of 3,5-diaryl-4,5-dihydropyrazole10-16. In addition, pyrazole derivatives substituted with thiazolyl group were reported to posses appreciable antibacterial as well as fungicidal activity14-16.

In the light of these findings, we aimed to synthesis new 3,5-diaryl-4,5-dihydropyrazoles substituted at position 1 with carboxamide or carbothioamide groups as well as cyclization of the carbothioamide group into thiazole ring. Finally some of the synthesized compounds were tested for their possible antimicrobial activity.

EXPERIMENTAL

Melting points were determined on a Griffin apparatus and were uncorrected. IR spectra were determined as KBr discs on Shimadzu IR 435 spectrophotometer and values were represented in cm⁻¹. The 1H NMR were carried out on Varian Gemini 200 MHz spectrophotometer, Microanalytical center, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on Hewlett Packard 5988 spectrometer, Microanalytical center, Cairo University, Cairo, Egypt. Elemental analyses were carried out at the Microanalytical center, Cairo University, Cairo, Egypt, and at the Microanalytical laboratory, National Research Center, Cairo, Egypt. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F 254 that were visualized using UV lamp and iodine vapor. The solvent system used in TLC was benzene : methanol : chloroform [ 9 : 3 : 1.5 ].
1,3-Diaryl-2-propen-1-ones 1a-e were prepared according to the literature \(^{17-20}\)

**General procedure for the synthesis of 3,5-diaryl-4,5-dihydro pyrazole-1-carboxamides 2a-c and 3,5-diaryl-4,5-dihydropyrazole-1-carbothioamides 3a,b**

A mixture of the respective chalcone analogues 1a-e (0.005 mol), semicarbazide HCl or thiosemicarbazide (0.005 mol) and NaOH (0.5 g, 0.0125 mol) was heated under reflux in absolute ethanol (12.5 ml) for 12 h. The reaction mixture was cooled and poured into an ice cold water. The solid separated was filtered, dried and crystallized from the suitable solvent.

**5-(2-Chlorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carboxamide (2a)** (crystallized from ethyl acetate); Yield: 78%; mp: 248-249 °C; IR (cm\(^{-1}\)): 3500, 3300 (NH\(_2\)), 1690 (C=O). \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.01 (dd, 1H, HA, J\(_{AB}\)=17.85 Hz, J\(_{AX}\)=5.50 Hz), 4.92 (dd, 1H, HB, J\(_{AB}\)=17.85 Hz, J\(_{BX}\)=12.46 Hz), 5.65 (dd, 1H, HX, J\(_{AX}\)=5.50 Hz, J\(_{BX}\)=12.46 Hz), 6.43 (br s, 2H, NH\(_2\), D\(_2\)O exchangeable), 7.11 -8.23 (m, 9 H, Ar-H); Anal. Calcd for C\(_{16}\)H\(_{14}\)ClN\(_3\)O: C, 64.11; H, 4.70; N, 14.01. Found: C, 64.40; H, 4.68; N, 14.32.

**5-(2-Furyl)-3-phenyl-4,5-dihydropyrazole-1-carboxamide (2b)** (crystallized from toluene); Yield: 59%; mp: 191-192 °C; IR (cm\(^{-1}\)): 3400, 3300 (NH\(_2\)), 1660 (C=O); \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.22 (dd, 1H, HA, J\(_{AB}\)=17.60 Hz, J\(_{AX}\)=5.30 Hz), 3.71 (dd, 1H, HB, J\(_{AB}\)=17.60 Hz, J\(_{BX}\)=12.15 Hz), 5.62 (dd, 1H, HX, J\(_{AX}\)=5.30 Hz, J\(_{BX}\)=12.15 Hz), 6.23 (dd, 1H, 4-H of furyl), 6.43 (br s, 2H, NH\(_2\), D\(_2\)O exchangeable), 7.39 -8.00 (m, 7 H, Ar- H); MS (EI, m/z(%)): 255 [M+, 40.95]; Anal. Calcd for C\(_{14}\)H\(_{13}\)N\(_3\)O\(_2\): C, 65.87; H, 5.13; N, 16.46. Found: C, 65.92; H, 5.32; N, 16.18.

**3-(4-Bromophenyl)-5-(2-furyl)-4,5-dihydropyrazole-1-carboxamide (2c)** (crystallized from ethyl acetate); Yield: 83%; mp: 191-192 °C; IR (cm\(^{-1}\)): 3400, 3300 (NH\(_2\)), 1690 (C=O). \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.35 (dd, 1H, HA, J\(_{AB}\)=17.52 Hz, J\(_{AX}\)=5.20 Hz), 3.76 (dd, 1H, HB, J\(_{AB}\)=17.52 Hz, J\(_{BX}\)=11.94 Hz), 5.52 (dd, 1H, HX, J\(_{AX}\)=5.20 Hz, J\(_{BX}\)=11.94 Hz), 6.36 (dd, 1H, 4-H of furyl), 6.51 (br s, 2H, NH\(_2\), D\(_2\)O exchangeable), 7.52 -7.82 (m, 6 H, Ar-H); Anal. Calcd for C\(_{14}\)H\(_{12}\)BrN\(_3\)O\(_2\): C, 50.31; H, 3.61; N, 12.57. Found: C, 50.14; H, 3.41; N, 12.41.

**3-(4-Bromophenyl)-5-(2-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (3a)** (crystallized from ethyl acetate); Yield: 79%; mp: 232-233 °C; IR (cm\(^{-1}\)): 3500, 3300 (NH\(_2\)), 1350 (C=S); \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.10 (dd, 1H, H\(_X\), J\(_{AX}\)=18.21 Hz, J\(_{BX}\)=3.08 Hz), 4.02 (dd, 1H, H\(_B\), J\(_{AB}\)=18.21 Hz, J\(_{BX}\)=11.66 Hz), 6.12 (dd, 1H, H\(_X\), J\(_{AX}\)=3.08 Hz, J\(_{BX}\)=11.66 Hz), 6.93-7.86 (m, 8H, Ar-H), 8.10-8.21 (two s, 2H, NH\(_2\), D\(_2\)O exchangeable); Anal. Calcd for C\(_{16}\)H\(_{15}\)BrCIN\(_2\)S: C, 48.68; H, 3.31; N, 10.64. Found: C, 49.05; H, 3.23; N, 10.70.

**3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (3b)** (crystallized from ethyl acetate); Yield: 78%; mp: 260-261 °C; IR (cm\(^{-1}\)): 3400, 3300 (NH\(_2\)), 1350 (C=S); \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.21 (dd, 1H, H\(_X\), J\(_{AX}\)=18.11 Hz, J\(_{BX}\)=3.21 Hz), 3.86 (dd, 1H, H\(_B\), J\(_{AB}\)=18.11 Hz, J\(_{BX}\)=11.30 Hz), 6.04 (dd, 1H, H\(_X\), J\(_{AX}\)=3.21 Hz, J\(_{BX}\)=11.30 Hz), 7.02-7.85 (m, 8H, Ar-H), 8.10-8.21 (two s, 2H, NH\(_2\), D\(_2\)O exchangeable); MS (EI, m/z(%)): 397 [(M+4)+, 6.97%], 395 [(M+2)+, 20.70%], 393 [M+, 15.83%], 60 [(CSNH\(_2\))+, 77.93%]; Anal. Calcd for C\(_{16}\)H\(_{13}\)BrClN\(_3\)S: C, 48.68; H, 3.31; N, 10.64. Found: C, 49.00; H, 3.62; N, 10.32.

**General procedure for the synthesis of 1-[3,5-diaryl-4,5-dihydropyrazol-1-yl]-4-(4'-bromophenyl)thiazoles 4a,b**

To a solution of the respective 2-pyrazoline-carbothioamides 3a,b (0.002 mol) in absolute ethanol (6 ml), 4-bromophenacyl bromide (0.56 g, 0.002 mol) was added and the mixture was heated under reflux for 1.5 h. On cooling, the separated solid was filtered, dried and crystallized from the suitable solvent.

**1-[3-(4-Bromophenyl)-5-(2-chlorophenyl)-4,5-dihydropyrazol-1-yl]-4-(4'-bromophenyl)thiazole (4a)** (crystallized from toluene); Yield: 61%; mp: 238-239 °C; IR (cm\(^{-1}\)): 1590 (C=N); \(^{1}H\) NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.30 (dd, 1H, H\(_X\), J\(_{AX}\)=18.10 Hz, J\(_{BX}\)=3.20 Hz), 3.71 (dd, 1H, H\(_B\), J\(_{AB}\)=18.10 Hz, J\(_{BX}\)=11.16 Hz), 6.00 (dd, 1H, H\(_X\), J\(_{AX}\)=3.20 Hz, J\(_{BX}\)=11.16 Hz), 6.93-7.85 (m, 8H, Ar-H), 8.10-8.21 (two s, 2H, NH\(_2\), D\(_2\)O exchangeable); Anal. Calcd for C\(_{24}\)H\(_{18}\)BrN\(_5\).S; C, 50.31; H, 3.61; N, 12.57. Found: C, 50.14; H, 3.41; N, 12.41.
1-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl]-4-(4'-bromophenyl)thiazole (4b)

(recrystallized from toluene); Yield: 79%; mp: 218-219 °C; IR (cm⁻¹): 1590 (C=N); 1H NMR (200 MHz, DMSO-d₆) δ ppm 3.33 (dd, 1H, HA, J_{AB}=18.17 Hz, J_{AX}=3.33 Hz), 3.71 (dd, 1H, HB, J_{AB}=18.17 Hz, J_{BX}=11.48 Hz), 6.12 (dd, 1H, HX, J_{AX}=3.33 Hz, J_{BX}=11.48 Hz), 7.30-8.09 (m, 13 H, Ar-H); Anal. Calcd for C_{24}H_{16}Br_{2}ClN_{3}S: C, 50.24; H, 2.81; N, 7.32. Found: C, 50.19; H, 3.01; N, 7.11.

1-[3-(4-Bromophenyl)-5-(2-chlorophenyl)-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydrothiazole (5a)

(crystallized from ethanol); Yield: 66%; mp: 294-296 °C; IR (cm⁻¹): 1690 (C=O); ¹H NMR (200 MHz, DMSO-d₆) δ ppm 3.37 (dd, 1H, HA, J_{AB}=17.67 Hz, J_{AX}=4.30 Hz), 3.94 (s, 2H, COCH₂-S), 4.03 (dd, 1H, HB, J_{AB}=17.67 Hz, J_{BX}=11.56 Hz), 6.00 (dd, 1H, HX, J_{AX}=4.30 Hz, J_{BX}=11.56 Hz), 7.24 -7.80 (m, 8 H, Ar-H); Anal. Calcd for C_{18}H_{13}BrClN_{3}OS: C, 49.73; H, 3.01; N, 9.66. Found: C, 49.73; H, 2.80; N, 9.98.

1-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydrothiazole (5b)

(recrystallized from ethyl acetate); Yield: 66%; mp: 276-277 °C; IR (cm⁻¹): 1700 (C=O); ¹H NMR (200 MHz, DMSO-d₆) δ ppm 3.45 (dd, 1H, HA, J_{AB}=18.32 Hz, J_{AX}=4.28 Hz), 3.92 (s, 2H, COCH₂-S), 4.16 (dd, 1H, HB, J_{AB}=18.32 Hz, J_{AX}=11.54 Hz), 5.83 (dd, 1H, HX, J_{AX}=4.28 Hz, J_{BX}=11.54 Hz), 7.26 -7.80 (m, 8 H, Ar-H); MS (EI, m/z(%)): 437 [(M+4)⁺, 7.85%], 435 [(M+2)⁺, 26.42%], 433 [(M⁺, 18.51%); Anal. Calcd for C_{18}H_{13}BrClN_{3}OS: C, 49.73; H, 3.01; N, 9.66. Found: C, 50.02; H, 3.45; N, 9.41.

General procedure for the synthesis of 1-[3,5-diaryl-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydrothiazoles 5a,b

A mixture of the respective 2-pyrazoline-1-carbothioamides 3c,e (0.002 mol) and ethyl chloroacetate (0.49 g, 0.4 ml, 0.004 mol) in glacial acetic acid (10 ml) was heated under reflux for 12 h. The reaction mixture was cooled, poured gradually into crushed ice, and left overnight. The resulting solid product was filtered and crystallized from the suitable solvent.

1-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydrothiazole (5b)

(recrystallized from ethyl acetate); Yield: 66%; mp: 276-277 °C; IR (cm⁻¹): 1700 (C=O); ¹H NMR (200 MHz, DMSO-d₆) δ ppm 3.45 (dd, 1H, HA, J_{AB}=18.32 Hz, J_{AX}=4.28 Hz), 3.92 (s, 2H, COCH₂-S), 4.16 (dd, 1H, HB, J_{AB}=18.32 Hz, J_{AX}=11.54 Hz), 5.83 (dd, 1H, HX, J_{AX}=4.28 Hz, J_{BX}=11.54 Hz), 7.26 -7.80 (m, 8 H, Ar-H); MS (EI, m/z(%)): 437 [(M+4)⁺, 7.85%], 435 [(M+2)⁺, 26.42%], 433 [(M⁺, 18.51%); Anal. Calcd for C_{18}H_{13}BrClN_{3}OS: C, 49.73; H, 3.01; N, 9.66. Found: C, 50.02; H, 3.45; N, 9.41.

Preliminary Antimicrobial Testing

Test organisms

Compounds 2c, 4b and 5a were screened for their antimicrobial activity using: Staphylococcus aureus and Bacillus subtilis as representatives of Gram positive bacteria, Escherichia coli and Pseudomonas aeruginosa as representatives of Gram negative bacteria and Candida albicans as representative of fungi.

Culture media

nutrient broth, Sabouraud 's broth and agar.

Method: Agar plate diffusion technique (21)

Agar plates containing 15 ml of agar medium [nutrient agar for bacteria and Sabouraud’s dextrose for fungi] were seeded with 0.2 ml of 18 hours broth culture of each organism.

Sterile filter paper discs (6 mm in diameter) were impregnated each with 10 µL of a saturated solution of the tested compound in DMF and allowed to air dry. The discs were then placed onto the surface of agar plates and incubated at 37°C for 48 hours. Control discs impregnated with DMF were used to determine the solvent activity. Antimicrobial activity of the test compounds was evaluated by measuring the zone of growth inhibition against the test organisms.

RESULTS

The obtained results were presented in Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Activity</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>2c, 4b, 5a</td>
<td>Moderate</td>
<td>Gram positive bacteria</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The synthesis of the target compounds is outlined in Scheme 1. The chalcone analogues 1a-e were synthesized by reported methods (17-20) and reacted with semicarbazide or
thiosemicarbazide in ethanolic NaOH to afford 3,5-diaryl-4,5-dihydropyrazole-1-carboxamides 2a-c and 1-carbothioamides 3a,b, respectively.

The structure conformation of compounds 2a-c and 3a,b was supported by elemental analyses, IR, 1-H NMR as well as mass spectra. The IR spectra of compounds 2a-c showed a forked band at 3500-3300 cm⁻¹ corresponding to NH₂ group and a band at 1690-1660 cm⁻¹ assigned to the carbonyl group. While, the IR spectra of compounds 3a,b demonstrated a forked band at 3500-3300 cm⁻¹ corresponding to NH₂⁻ group and a band at 1350 cm⁻¹ corresponding to C=S group.

The formation of 2-pyrazoline ring was confirmed by the appearance of ABX system in 1-H NMR due to geminal-vicinal coupling between protons H₄ and H₅ at C-4 and H₅- at C-5. H₅ which appears as doublet of doublets around δ 3-3.4 ppm is the proton trans to H₅⁻ and geminal to H₅ (J₅₆=17-18 Hz, J₆₇=3-6 Hz). H₅⁻ is the proton cis and vicinal to H₅⁻ and appears as doublet of doublets around δ 3.7-4.9 ppm. While, H₅⁻ appears as doublet of doublets around δ 5.5-6.4 ppm (J₅₆=11-12 Hz) (22, 23). Compounds substituted with 2-furyl ring showed an additional doublet of doublets around δ 6.2-6.3 characteristic to 4-CH (H₄) of 2-furyl ring (AMX system)²⁴. Beside these signals, the amino protons appeared as an exchangeable broad singlet signal at δ 6.4-6.5 in compounds 2a-c and as two exchangeable signals at δ 8.1-8.2 in compounds 3a,b.

The mass spectrum of 2b showed a molecular ion peak at m/z 255. (chart 1 showed the possible fragmentation pattern of 2b). While, the mass spectrum of 3b displayed molecular ion peaks at m/z 393, 395 and 397 corresponding to M, M+2 and M+4 in the ratio of 100: 130: 44 (BrCl pattern) (25).

1-[3,5-Diaryl-4,5-dihydropyrazol-1-yl]-4-(4'-bromophenyl)-thiazoles 4a,b were obtained via reacting the appropriate 2-pyrazoline-1-carbothioamides 3a,b with 4-bromophenacyl bromide in boiling ethanol. The IR spectra of compounds 4a,b lacked the presence of absorption bands due to C=S group and NH₂⁻ group. On parallel line, 1-H NMR spectra of 4a and 4b revealed the disappearance of signals at δ 8.1-8.2 corresponding to NH₂⁻ protons.

1-[3,5-Diaryl-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydrothiazoles 5a,b were synthesized via reacting pyrazoline-1-carbothioamides 3a,b with ethyl chloroacetate in acetic acid. The IR spectra of 5a,b indicated the disappearance of absorption bands of NH₂⁻ and C=S groups. Meanwhile, a band at 1700-1690 cm⁻¹ corresponding to the carbonyl group appeared.

Further evidence for this structure was obtained from 1-H NMR spectra for 5a and 5b that showed a singlet signal at δ 3.9 corresponding to CH₂⁻ group of thiazolidone ring and the disappearance of the exchangeable signals of the amino group protons.

In addition, the mass spectrum of 5b showed a molecular ion peak at m/z 433, 435 and 437 corresponding to M, M+2 and M+4 in the ratio of 100: 142: 42 (BrCl pattern) (25).

### Table 1: Results of the antimicrobial testing for some of the synthesized compounds

<table>
<thead>
<tr>
<th>Comp. No</th>
<th>Zone of Inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>2c</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>-</td>
</tr>
<tr>
<td>5a</td>
<td>-</td>
</tr>
<tr>
<td>DMF</td>
<td>-</td>
</tr>
<tr>
<td>Amoxacillin</td>
<td>8</td>
</tr>
<tr>
<td>Propenicillin</td>
<td>8</td>
</tr>
</tbody>
</table>
Scheme 1:

Chart 1: The possible fragmentation pattern of 2b
The antimicrobial activities of some of the synthesized compounds were determined by Agar plate diffusion technique\textsuperscript{21}. The compounds were evaluated for antimicrobial activity against bacteria (both Gram positive and Gram negative strains) and fungi. The antibiotics amoxacillin\textsuperscript{®} and propenicillin\textsuperscript{®} were used as reference substances for comparison. DMF was used as control and to test the solvent activity. The diameter of the inhibition zone around each disc was measured in mm and the results were presented in Table 1.

It has been observed that Compounds 2c and 5a showed moderate activity only against Gram positive bacteria. None of the tested compounds showed antibacterial activity against Gram negative strain or antifungal activity against Candida albicans.

CONCLUSION

The objective of the present study was to synthesize and investigate the antimicrobial activities of new series of 3,5-diarylprazolines and pyrazolylthiazoles. Amongst the tested compounds, 2c and 5a showed moderate antibacterial activity against Gram-positive bacteria.

ACKNOWLEDGEMENTS

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REFERENCES