SYNTHESIS OF SOME 4-BENZYL-1-SUBSTITUTED PIPERAZINE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AND ANTIQUELIGEN AGENTS

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Several derivatives of N1-(4-benzyl-l-piperazinylacetato)-N4-substituted thiosemicarbazides were prepared, some of the corresponding cyclized derivatives, 3-mercapto-4-phenyl-5-(4-benzyl-l-piperazinylmethyl)-1,2,4-triazole 
and 2-(4-benzyl-l-piperazinylmethyl)-5-phenylaminoo-1,3,4-oxadiazole were also prepared and characterized by elemental analysis, infra red, and nuclear magnetic resonance (1H-NMR) spectra. The compounds were evaluated for their in vitro antimicrobial activity, the 4-benzoyl thiosemicarbazide derivative 3e and its cyclized derivatives 4b and 5b were the most active. Most of the compounds were active in the Brine Shrimp Lethality Test (BST), compound 3e which was the most active on the BS test was also found to possess a significant cytotoxicity against several tumor cell lines.

Keywords: Piperazine derivatives; Synthesis; Antimicrobial; Anticancer agents

Introduction

Many compounds related to substituted thiosemicarbazide and the corresponding cyclized 1,2,4-oxadiazole derivatives having different functional groups have been shown to possess different biological activities including antineoplastic and antibacterial activities (1-3). The piperazine derivatives are known for their antihelmintic activity (4), besides, the piperazine nucleus was found incorporated into a large number of drug classes including antimicrobial agents (5). It seems that these moieties are useful in imparting biological activities, which suggested the synthesis of other derivatives containing both the thiosemicarbazide, the cyclized 1,2,4-triazole or cyclized 1,3,4-oxadiazole and the piperazine nucleus with the hope that incorporation of these moieties might result in biologically active agents.

Materials and Methods

a. Chemistry

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. The IR spectra (KBr) were determined on a Shimadzu spectrophotometer IR-435. The proton nuclear magnetic resonance (1H-NMR) spectra were recorded on a Bruker WP 80 pulse spectrometer. The elemental analysis were provided by M.H.W Laboratories (Phoenix, AZ) and the Mic-

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Table 1. N1-(4-benzyl-1-piperazinylaceto)-N4-substituted thiosemicarbazides (3a-e), their cyclized derivatives 1,2,4-triazole (4b,e) and 1,3,4-oxadiazoles (5b,e)

<table>
<thead>
<tr>
<th>No</th>
<th>R</th>
<th>mp. (°C)</th>
<th>Yield/RS*</th>
<th>Molecular Formula</th>
<th>Analysis, %, Calcld./found</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
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<tr>
<td>3a</td>
<td>n-C₄H₉</td>
<td>127-128</td>
<td>86/B</td>
<td>C₁₈H₂₀N₅O₅S</td>
<td>59.47</td>
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<tr>
<td>3b</td>
<td>C₆H₅</td>
<td>166-167</td>
<td>68/Ch</td>
<td>C₂₀H₂₂N₅O₅S</td>
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<tr>
<td>3c</td>
<td>C₆H₄CH₃ (meta)</td>
<td>167-168</td>
<td>92/Ch:E</td>
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<tr>
<td>3d</td>
<td>CH₂-C₆H₅</td>
<td>159-160</td>
<td>88/Et</td>
<td>C₂₁H₂₇N₅O₅S</td>
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<td>3e</td>
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<td>90/Ch:E</td>
<td>C₂₁H₂₅N₅O₂S</td>
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<tr>
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<td>184-185</td>
<td>64/B</td>
<td>C₂₀H₂₀N₅S</td>
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<tr>
<td>4e</td>
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<td>51/Ch:E</td>
<td>C₂₁H₂₁N₅S</td>
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<tr>
<td>5b</td>
<td>C₆H₅</td>
<td>164-165</td>
<td>52/Ch:E</td>
<td>C₂₀H₂₃N₅O</td>
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<td>84/E:B</td>
<td>C₂₁H₂₃N₅O₂</td>
<td>68.77</td>
</tr>
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</table>

RS*: recrystallization solvent, B: benzene, Ch: chloroform, E: ether, Et: ethanol

3. N1-(4-benzyl-1-piperazinyl) aceto-N4-substituted thiosemicarbazides (3a-e, Table 1)

To a solution of 2 (1.24 g, 3 mmole) in chloroform (25 ml), the appropriate isothiocyanate (3 mmole) was added, and the mixture was heated under reflux for 0.5-2 hours. The solvent was evaporated and the residue was recrystallized from the appropriate solvent. The pure products were obtained in 68-92% yield: IR (cm⁻¹) for 3b: 3110-3320 (N-H) and 1668 (C=O); 1235, 1265, 1420, 1460 (C=S); 1H-NMR (CDCl₃) δ 2.61 (s,8H), 3.15 (s,2H), 3.52 (s,2H), 7.29 (m,5H), 7.3-7.5 (m,5H), 8.5 (broad, s,1H).

4. 3-Mercapto-4-phenyl-5-(4-benzyl-1-piperazinylmethyl)-1,2,4-triazole (4b,e)

A solution of 3b or 3e (2 mmole) in sodium hydroxide (25 ml, 0.1 N) was heated under reflux for one hour. The solution was cooled at room temperature and the pH was adjusted with 0.1 N HCl to 6.5. The product formed was filtered and recrystallized to give the pure product: 4b, IR (cm⁻¹) 2680 (SH)and 1595 (C≡N); 1H-NMR (CDCl₃) δ 2.40 (s,8H), 3.36 (s,2H), 3.49 (s,2H), 7.2-7.3 (m,5H), 7.4-7.5 (m,5H).

5. 2-(4-benzyl-1-piperazinylmethyl)-5-phenylamino-1,3,4-oxadiazole (5b,e)

To a solution of 3b or 3e (25 mmole) in anhydrous ethanol (20 ml), dicyclohexylcarbodiimide (0.77 g, 3.75 mmole) was added and the mixture was heated under reflux for two hours. The reaction mixture was cooled, the solvent was evaporated under vacuum and the crude product was recrystallized: 5b, IR (cm⁻¹) 3275 (NH), 1620 and 1575 (C≡N), 1290 (C=O-C); 1H-NMR (CDCl₃) δ 2.55 (s,4H), 2.59 (s,4H), 3.51 (s,2H), 3.74 (s,2H), 7.25-7.3 (m,5H), 7.3-7.6 (m,5H).

b. Antimicrobial Activity

Antibacterial activities of the compounds were tested against two Gram-positive microorganisms (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923) and two Gram-negative microorganisms (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa PA 0303) by macrodilution method (7) in Brain Heart Infusion Broth (BHI, Difco). Nalidixic acid (pharmaceutical grade) was used as an antibacterial reference substance and was kindly provided by Dar Al-Dawa Development and Investment Company, Na'ur, Jordan.

Antifungal activities of the compounds were tested against yeast-like fungi (Candida albicans IGR 66-hospital isolate, Institute Gustave-Roussy, France) by macrodilution method (7) in Sabouraud's Liquid medium (SLM, Oxoid, England). Microazole nitrate (pharmaceutical grade) was used as an antifungal reference substance and was kindly provided by The Middle East Pharmaceutical and Chemical Industries and Medical Appliances Company, Amman, Jordan.

Stock solutions of the compounds were prepared in dimethyl sulfoxide: absolute ethanol mixture (1:1) at 4 mg/ml concentration. The stock solutions were two-fold diluted (2000 to 0.49 μg/ml) in BHI broth.
and nuclear magnetic resonance.

Evaluation of the compounds for their antimicrobial activities (Table 2) showed that, the most active compounds (3e and 5e) have antifungal activities at MIC value of 125 μg/ml. Compared to miconazole nitrate which was used as a reference substance for the antifungal activity (11), with an MIC value of 0.49 μg/ml, it is obvious that these compounds have weak activities. The compound which showed the highest activity against *B. subtilis* was 3e as it showed an MIC value of 31.25 μg/ml. Compound 3e and the corresponding cyclized 1,2,4-triazole, 4e and 1,3,4-oxadiazole, 5e, derivatives showed the same MIC value of 125 μg/ml against *S. aureus*.

Compounds 4e and 5e were the most active against *E. coli* and *P. aeruginosa*, and showed MIC values of 125 μg/ml. Comparing the antibacterial activity of the most active compounds in this series (4e and 5e) with the bactericidal drug nalidixic acid (11), it was found that they have comparable activities against three of the four tested bacterial strains and nalidixic acid was found to be 16 times more active against *E. coli*. These results indicate that the presence of the electron withdrawing carbonyl group in compound 3e increases significantly the *in vitro* antimicrobial activity against *B. subtilis*, *S. aureus*, and *C. albicans* by 32, 8 and 4 folds respectively compared with compound 3b. The cyclized derivatives of compound 3e, the triazole 4e and the oxadiazole 5b, improved the antibacterial activity against *E. coli* and *P. aeruginosa* by 4 and 8 folds respectively.

The brine shrimp bioassay was developed by Meyer et al (8) to detect the cytotoxic effects of pharmacologically active materials. Acrosol et al found the BS cytotoxicities and tests which are used to screen anticancer activity. In this study the BS bioassay was used as a general screening test for the cytotoxicity of synthetic compounds. Compounds with LC50 of more than 1000 μg/ml were considered inactive (9). Only compounds 3b and 2 were found inactive. The most active compound in this bioassay was compound 3e with LC50 of 20.8 mg/ml (Table 2). This compound was evaluated further using
three tumor cell lines at the Purdue Cancer Center and was found to possess a significant cytotoxicity against all cell lines. The activity is considered significant when the ED$_{50}$ is 10 $\mu$g/ml or less (10). The ED$_{50}$ of compound 3e were 3.55 $\mu$g/ml (A-549, Human Lung Cancer); 5.52 $\mu$g/ml (MCF-7, Human Breast Cancer) and 2.18 $\mu$g/ml (HT-29, Human Colon Cancer). In comparison between the antibacterial and the BS test results it was noticed that two of the three most active compounds in the antimicrobial tests 3e and 4e were also the most active in the BS test.

The antimicrobial and anticancer activities of compounds 3e and the antimicrobial activities of its cyclized derivatives 4e and 5e suggest that structural activity studies on these compounds might prove to be useful.

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References

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