

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

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Several derivatives of N1-(4-benzyl-1-piperazinylaceto)-N4-substituted thiosemicarbazides were prepared, some of the corresponding cyclized derivatives, 3-mercapto-4-phenyl-5-(4-benzyl-1-piperazinylmethyl)-1,2,4-triazole and 2-(4-benzyl-1-piperazinylmethyl)-5-phenylamino-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 antihelminthic 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-

roanalytical Unit (Cairo University, Cairo, EGYPT).

Synthesis

1. Methyl(4-benzyl-1-piperazinyl)acetate(1)

To a solution of 1-benzyl piperazine (35.2 g, 0.2 mole) in tetrahydrofuran (200 ml), potassium carbonate (27.6 g, 0.2 mole) was added followed by the addition of methyl bromoacetate (30.6 g, 0.2 mole) in tetrahydrofuran (50 ml) dropwise with stirring over a period of fifteen minutes at room temperature, and the reaction mixture was heated under reflux for an additional one hour. The solution was cooled, filtered, and the solvent was evaporated. The crude product was recrystallized from dichloromethane: ether mixture to give the pure compound in 82% yield: IR (cm⁻¹) 1735 (-C=O); ¹H-NMR (CDCl₃) δ 3.04 (s,8H), 3.47 (s,2H), 3.74 (s,3H), 4.37 (s,2H), 7.5-7.6 (m,5H).

2. 4-benzyl-1-piperazinylacetohydrazide (2)

To a stirred solution of **1** (14.88 g, 7 mmole) in ethanol (50 ml), hydrazine hydrate (7.0 g, 14 mmole) was added, the mixture was refluxed for one hour, an additional amount of hydrazine hydrate (7.0 g, 14 mmole) was added, and the reaction mixture was refluxed for an additional one hour. The reaction mixture was cooled, the solvent evaporated under vacuum, the residue was dissolved in chloroform (50 ml) and washed three times with water (50 ml each time). The chloroform layer was dried over magnesium sulfate, filtered, and the solvent was evaporated. The crude product was recrystallized from chloroform: ethanol mixture to give the pure product in 56% yield: mp: 103-104°C (reported 103-104°C (6)); IR (cm⁻¹) 3400, 3290 (N-H) and 1680 (C=O); ¹H-NMR (CDCl₃) δ 2.52 (s,8H), 3.07 (s,2H), 3.52 (s,2H), 3.8 (s,2H), 7.3-7.6 (m, 5H), 8.1 (s,1H).

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

No	R	mp. °C	Yield/RS ^a	Molecular Formula	Analysis %, Calcd./found		
					C	H	N
3a	n-C ₄ H ₉	127-128	86/B	C ₁₈ H ₂₉ N ₅ OS	59.47	8.04	19.26
					59.30	7.80	18.90
3b	C ₆ H ₅	166-167	68/Ch	C ₂₀ H ₂₅ N ₅ OS	62.63	6.57	18.26
					62.40	6.30	18.50
3c	C ₆ H ₄ -CH ₃ (meta)	167-168	92/Ch:E	C ₂₁ H ₂₇ N ₅ OS	63.45	6.85	17.62
					63.40	6.90	17.40
3d	CH ₂ -C ₆ H ₅	159-160	88/Et	C ₂₁ H ₂₇ N ₅ OS	63.45	6.85	17.62
					63.40	6.80	-----
3e	CO-C ₆ H ₅	174-175	90/Ch:E	C ₂₁ H ₂₅ N ₅ O ₂ S	61.29	6.12	17.02
					61.20	5.80	17.20
4b	C ₆ H ₅	184-185	64/B	C ₂₀ H ₂₃ N ₅ S	65.70	6.34	19.00
					65.70	6.34	19.00
4e	CO-C ₆ H ₅	151-153	51/Ch:E	C ₂₁ H ₂₃ N ₅ OS	64.12	5.85	17.81
					64.70	5.98	-----
5b	C ₆ H ₅	164-165	52/Ch:E	C ₂₀ H ₂₃ N ₅ O	68.77	6.59	20.06
					68.29	6.71	19.88
5e	CO-C ₆ H ₅	94-95	84/E:B	C ₂₁ H ₂₃ N ₅ O ₂	66.84	6.10	18.57
					66.70	6.53	18.29

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

3. N1-(4-benzyl-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, 37.5 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). Miconazole 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

tubes for bacteria and SLM tubes for fungi. 0.1 microorganism suspensions at 10^6 CFU/ml concentration were inoculated into all serial tubes. The tubes were incubated at 37°C for 18 hours. The lowest concentration of the compounds that completely inhibits macroscopic growth was controlled and MIC reported. Dimethyl sulfoxide: absolute ethanol mixture (1:1) and its dilutions were verified as a control in this test.

c. Brine Shrimp Bioassay

This assay was performed as described by Meyer *et al* (8). The brine shrimp eggs (Living World, Metaframe Inc., Elmwood Park, NJ, USA) were hatched in artificial sea water prepared from a commercial salt mixture (Instant Oceans, Aquarium System Inc. Mentor, OH, USA). The samples were prepared by dissolving 10 mg of the compound in 2 ml methanol. Appropriate amounts from the final solution were transferred to vials and dried. The bioassay was performed by transferring 10 shrimps to the vials using a disposable Pasteur pipette and artificial sea water was added to make the volume up to 5 ml. The nauplii were counted visually after 24 hours. The $LC_{50,s}$ and 95% confidence intervals were determined using the Probit Analysis Method described by Finney (9).

d. Cytotoxicity Tests

The cytotoxicity was determined in the Purdue Cell Culture Laboratory (Purdue Cancer Center, Purdue University, West Lafayette, IN, USA) against the Human Cell Lines, A-549 (Human Lung Carcinoma), MCF-7 (Human Breast Carcinoma) and HT-29 (Human Colon Adenocarcinoma) following protocols established by the National Cancer Institute (NCI) of the USA.

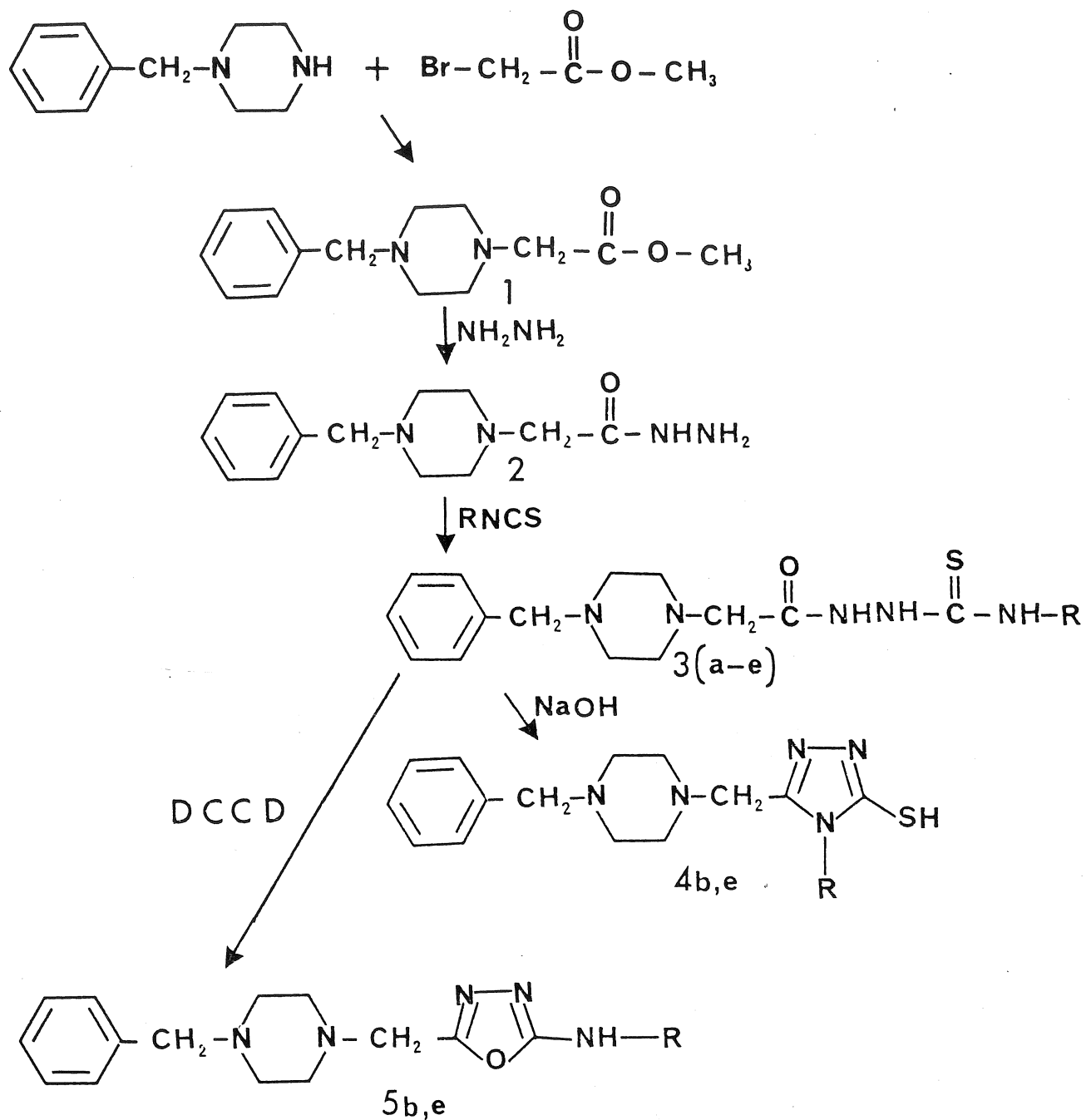
Results and Discussion

The compounds were prepared as shown in synthetic scheme, in accordance with the reported methods in the literature (1,2,4). 1-Benzyl piperazine was reacted with methyl bromoacetate and the resulting compound, **1**, was converted to 4 - benzyl - 1 - piperazinylacetohydrazide, **2**, by heating under reflux with hydrazine hydrate. Compound, **2**, was reacted with appropriate isothiocyanate to give the thiosemicarbazides, **3a-e**. Conversion of the thiosemicarbazide, **3b** and **3e**, to the corresponding cyclized 1,3,4-oxadiazole, **4b** and **4e**, was achieved by cyclodesulfurization using dicyclohexylcarbodiimide (DCCD) and refluxing the mixture for two hours in ethanol. The cyclized 1,2,4-triazole, **5b** and **5e** was prepared by refluxing the thiosemicarbazide, **3b** or **3e** with 0.1 N sodium hydroxide for one hour. The synthesized compounds were characterized by elemental analysis, infra red

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 μ g/ml. Compared to miconazole nitrate which was used as a reference substance for the antifungal activity (11), with an MIC value of 0.49 μ g/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 μ g/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 μ g/ml against *S. aureus*. Compounds **4e** and **5e** were the most active against *E. coli* and *P. aeruginosa*, and showed MIC values of 125 μ g/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. A correlation was found between 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 LC_{50} of more than 1000 μ g/ml were considered inactive (9). Only compounds 1 and 2 were found inactive. The most active compound in this bioassay was compound **3e** with LC_{50} of 20.8 mg/ml (Table 2). This compound was evaluated further using



Scheme

Table 2. Antimicrobial and brine shrimp bioassay results of benzyl piperazine derivatives

No	Microorganisms					Brine shrimp ^a LC ₅₀ in mg/ml (95% confidence interval)
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>C. albicans</i>	
	Activity µg/ml					
3a	1000	1000	500	1000	500	197(102-485)
3b	1000	1000	1000	1000	250	289 (102-970)
3d	2000	1000	250	1000	500	187(102-420)
3e	500	1000	31.25	125	125	20.8 (11-36.8)
4b	2000	2000	1000	2000	1000	924 (235-1106)
4e	125	125	125	125	125	54 (28-144)
5b	1000	500	1000	1000	250	21(8-81)
5e	125	125	62.5	125	125	139 (75-634)
NA ^b	7.8	125	250	62.5	-----	-----
MN ^c	-----	-----	----	-----	0.49	----

^a Compounds 1 and 2 were inactive while 3c activity was 39.2 (18.8-76.9); ^bNA: nalidixic acid; ^cMN: miconazole nitrate

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 ED₅₀ is 10 µg/ml or less (10). The ED₅₀s for compound 3e were 3.55 µg/ml (A-549, Human Lung Cancer); 5.52 µg/ml (MCF-7, Human Breast Cancer) and 2.18 µg/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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