Synthesis of 2,5-Disubstituted-1,4-benzoquinone Derivatives as Potential Antimicrobial and Cytotoxic Agents

Mohamad A. Hassan*[a], Ahmed O. Maslat[b], Mahmud Abussaud[b], Ibrahim Ch. Ahmed[c], and Ahmad S. AlKofahi[a]

[a] Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, and the [b] Department of Biology, Yarmouk University, Irbid, Jordan

[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexadria, Egypt

Key Words: *Benzoquinone derivatives; synthesis; antimicrobial; cytotoxicity; mutagenicity*

Summary

A number of 2,5-disubstituted-1,4-benzoquinone derivatives were prepared and characterized by elemental analysis, infrared (IR), nuclear magnetic resonance (^{1}H-NMR), and mass spectra (MS). These compounds and their synthetic precursors were evaluated for their *in vitro* antimicrobial and cytotoxic activity. The most potent antimicrobial compound was the thiadiazolyl derivative **4b,** which was 2- to 4 times more active than the antimicrobial drug sulfathiazole. All the tested compounds were active in the Brine Shrimp Lethality (BS) Test. Compound **4e** which was the most active in the BS test was also found to possess a significant cytotoxicity against two tumor cell lines. Some of the compounds were found to be mutagenic at relatively high concentration.

Introduction

The continuous development of bacterial resistance to the existing drugs has urged scientists to continue their search for new antibacterial drugs [1]. 1,3,4-Thiadiazoles, substituted at positions 2 and 5, and their open chain thiosemicarbazide analogues are an interesting group of compounds known for their wide range of biological activities including antimicrobial activities [2–6]. Quinones and hydroquinones are also found incorporated into a large number of drug classes, including anticancer [7] and antimicrobial [8] agents.

When allowed to react with a 1,4-benzoquinone, the sulfonamides and other bacteriostatic agents containing an amino group will give compounds considered to be vinylogous amides[9], which may undergo simple hydrolytic cleavage inside the microorganism [10]; bacteriostatic action can be achieved by the release of the amine, or they may have intrinsic antimicrobial activity.

In continuation of our work[11], a combination of 2-benzoylamino-1,3,4-thiadiazole and an aminoquinonyl derivatives at position 5[12,13] would be expected to result in biologically active agents.

Results and Discussion

The target compounds were synthesized by the reaction sequence shown in Scheme 1. Condensation of 2,5-dihydroxybenzoic acid hydrazide with benzoylisothiocyanate yielded N^1-(2,5-dihydroxybenzoyl)-N^4-benzoylthiosemicarbazide **1**. The thiosemicarbazide was cyclodehydrated with concentrated sulfuric acid [19] in absolute ethanol to yield the corresponding 1,3,4-thiadiazole **2**. The 2-substituted hydroquinone **2** was oxidized to the benzoquinone **3** with ferric chloride in dimethylformamide [13]. The reaction between the quinone and the amine [12, 20–22] results in the formation of the 2,5-disubstituted quinones **4a–g** (Table 1). The structure of each compound was confirmed by elemental analysis and spectroscopic techniques.

Table 1. 2-Arylamino-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinones (**4a–f**).

No	Aryl	% yield	Recryst. solvent[a]	Molecular formula[b]
4a	4-benzylpiperazinyl-	90.3	DM/Et	$C_{25}H_{21}N_5O_3S$
4b	4-[(N-(2-thiazolyl-aminosulfonyl)phenyl]-amino	85.0	DM/Et	$C_{24}H_{16}N_6O_5S_3$
4c	C_6H_5-	86.8	DM	$C_{21}H_{13}N_3O_3S$
4d	p-CH_3-C_6H_4-	87.0	DM/Et	$C_{22}H_{15}N_3O_3S$
4e	p-OH-C_6H_4-	78.6	THF/DM	$C_{21}H_{13}N_3O_4S$
4f	p-Br-C_6H_4-	80.9	DM/Et	$C_{21}H_{12}N_3O_3S$
4g	p-NO_2-C_6H_4-	64.2	DM/Et	$C_{21}H_{12}N_4O_5S$

[a] DM: dichloromethane; THF: tetrahydrofuran; Et: ethanol.
[b] Analyzed elements C, H, N.

The brine shrimp bioassay was used to detect the cytotoxic effect of pharmacologically active compounds [15]. The test was used in this study as a screening test for cytotoxic activities. Compounds with a median lethal concentration (LC_{50}) of more than 1000 μg/ml were considered inactive [16]. All the tested compounds were found active in the BS test. The most active compounds were **3, 4a, 4d, 4e**, and **4f** with LC_{50} of 7, 14, 0.019, 4.0×10^{-2}, and 3.7 μg/ml respectively (Table 2). These compounds were evaluated further using three tumor cell lines at the Purdue Cancer Center, and only compound **4e**, was found to possess significant cytotoxicity against two cell lines which was also the most active in the BS test (Table 2). The activity is considered significant when the median effective dose (ED_{50}) is 10 μg/ml or less[23]. The ED_{50}'s for compound **4e** were 16.48 μg/ml (A-549, Human Lung Cancer); 7.89 μg/ml (MCF-7, Human Breast Cancer); and 4.80 μg/ml (HT-29, Human Colon Cancer).

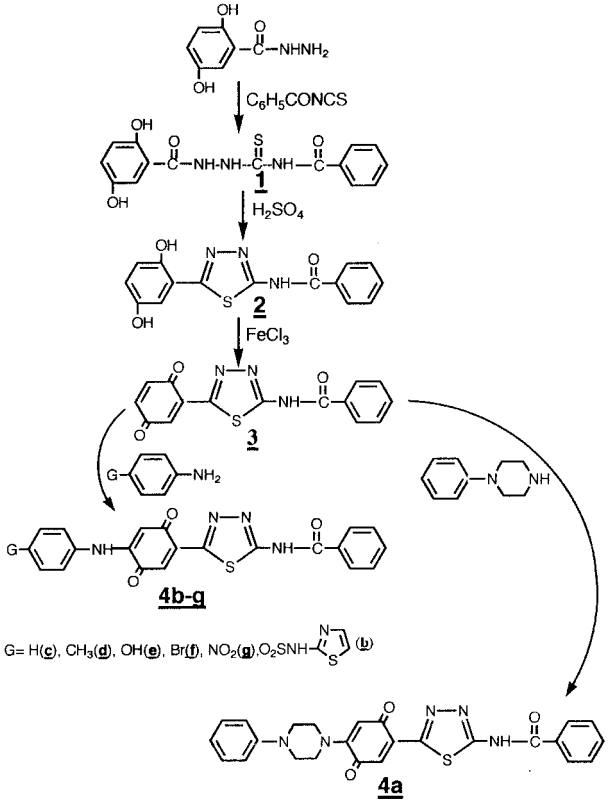
The antimicrobial testing showed that compounds **1**, **4b**, and **4g** have activities at a minimum inhibitory concentration (MIC) values of 250 μg/ml or less (Table 3). Compound **4b** has a weak antifungal activity with an MIC value of 31 μg/ml,

  0365-6233/98/1212/0385 $17.50 +.50/0

Table 2. Brine shrimp bioassay and cytotoxicity test results.

No.	Brine shrimp LC_{50} in μg/ml (95% Confidence Interval)	Cytotoxicity ED_{50} (μg/ml) A-549[a]	MCF-7[b]	HT-29[c]
1	26 (18–41)	–	–	–
2	31.5 (14–99)	–	–	–
3	7 (4–11)	38.8	>100	34.76
4a	14 (4.6–53)	65.38	>100	38.20
4b	189 (44–894)	–	–	–
4c	20.8 (5–242)	–	–	–
4d	0.019 (0.002–0.8)	36.31	76.22	13.60
4e	4.0×10^{-2} (0.01–0.578)	16.48	7.89	4.80
4f	3.7 (2.2–6.3)	95.94	>100	>100
4g	46.6 (13–1388)	–	–	–
AM[d]	–	4.36×10^{-3}	4.38×10^{-1}	2.45×10^{-2}

a) A-549 = Human lung carcinoma.
b) MCF-7 = Human breast carcinoma.
c) HT-29 = Human colon adenocarcinoma.
d) AM = Adriamycin.



Scheme 1

compared with the antifungal drug miconazole nitrate which was used as a reference substance[24] with an MIC value of 0.49 μg/ml. Compound **4b** showed good activity against both Gram negative and Gram positive microorganisms, compared with the bactericidal drug nalidixic acid [24]; they have comparable activities against the Gram positive microorganisms *Bacillus subtilis* and *Staphylococcus aureus* with the same MIC value of 62 μg/ml. Compound **4b** was found to be 8 times more active than nalidixic acid against *Pseudomonas aeruginosa*, and 8 times less active against *Escherichia coli*. Compound **1** was found to have good activity against *Staphylococcus aureus* with an MIC value of 8 μg/ml. Compared to the antimicrobial drug sulfathiazole, compound **4b** was found to be 2 to 4 times more active against all the tested microorganisms.

Table 3: Antimicrobial test results.

No.	Microorganism[a] *E. coli*	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*C. albicans*
1	250	250	2	8	250
4b	62	31	62	62	31
4g	250	250	2	250	250
Na[b]	8	250	62	62	31
TS[c]	125	125	250	250	125

a) The activity in μg/ml.
b) NA: nalidixic acid.
c) TS: N^1-(2-thiazolyl)sulfanilamide.

The results of the antimicrobial activity studies indicates that incorporation of the sulfa drug into the 2-substituted-1,4-benzoquinones might improve their antimicrobial activities, and further structural activity studies on these compounds might prove to be useful.

Compounds **1**, **4b**, and **4g** which showed promissing antimicrobial activities were evaluated further for their carcinogenic effect by evaluating their mutagenic action, since most carcinogens were also found to be mutagenic [25]. The results are presented in Table 4. The tested compounds were found to be mutagenic using the Ames test; however, the mutagenic activity of compounds **1** and **4g** were at maximum value at relatively high concentrations. They showed base pair substitution mutagenicity but no frameshift mutagenic activity, since the strains TA 100 and TA 1530, the only strains that gave positive results with the tested compounds, are known to detect base pair substitution mutagens only [18].

Table 4: Mutagenic activity of some compounds.

Compound No.	Strain	Concentration μg/plate[a]	No. of reverants per plate[b]
1	TA 1530	10	90
4b	TA 1530	1	360
4g	TA 100	26	600

a) The concentration at which the highest mutagenic activity was obtained.
b) An average of two experiments after substracting the spontaneous revertants.

From these results we suggest that further investigation using different systems must be made prior to any decision regarding their use in humans.

Acknowledgment

The authors wish to thank the Deanship of Scientific research at JUST and the Deanship of the Faculty of Pharmacy of Amman University, for providing necessary facilities. Special thanks to Professor J. L. Mclaughlin, School of Pharmacy, Purdue University, West Lafayette, Indiana, USA, for the anticancer testing.

Experimental

Chemistry

Material and Methods

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator F-254 (Sigma Chemical Co.). The infrared (IR) spectra were determined on a Shimadzu spectrophotometer IR-435 using the KBr disc technique. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker WP pulse spectrometer, and the chemical shifts are reported in δ units downfield from the internal reference tetramethylsilane. The mass spectra (MS) were measured on a 7070-E VG analytical mass spectrometer. The elemental analysis were provided by the Microanalytical unit (Alexandria University, Egypt). The analyzed elements were C, H, N.

Synthesis

2,5-Dihydroxybenzoic acid hydrazide was prepared from methyl 2,5-dihydroxybenzoate following the procedure reported in the literature[14].

*N^1-(2,5-dihydroxybenzoyl)-N^4-benzoylthiosemicarbazide (**1**)*

A mixture of 2,5-dihydroxybenzoic acid hydrazide (12.60 g, 75 mmol) in ethanol (75 ml) was heated under reflux for 0.5 h. The reaction mixture was allowed to cool to room temperature, the precipitate formed was filtered, and the product was washed with ethanol. The crude product was recrystallized from ethanol-chloroform mixture to give white pure crystals. Yield: 22.4g (90%).– Mp 229–231 °C.– TLC with methylene chloride: methanol (9.0:1.0,v/v).– R_f = 0.62.– IR (cm^{-1}) 3500 (OH),3280,3160 (NH), 1660, 1645, 1635 (C=O).– ^{1}H-NMR [D_6]DMSO (deuterated dimethyl sulfoxide), δ 6.86-8.05(m, 8 H, Ar-H), 7.37 (s,1 H, NH), 8.60 (s,1H,NH $_5$),9.16 (s,1 H, NH), 11.70 (s,1 H,OH), 11.85 (s,1H,OH). The NH and OH hydrogens are deuterium exchangable.– MS, *m/z* (%) 331 (M^+,46), 313 (13,M^+–H_2O), 168 (44, M^+–dihydroxycarbonyl), 137 (73[2,5-dihydroxycarbonyl cation]), 105 (100, benzoyl cation), 77 (67. phenyl cation).Anal. ($C_{15}H_{13}N_3O_4S\cdot 0.5H_2O$).

*2-Benzoylamino-5-(2,5-dihydroxyphenyl)-1,3,4-thiadiazole (**2**)*

To a stirred solution of **1** (9.00 g, 27 mmol) in absolute ethanol (200 ml), sulfuric acid (24 ml) was carefully added over a period of 15 min. The reaction mixture was left at room temperature for 20 h and then poured with stirring to an equal volume of ice-cold water. The precipitate formed was filtered, washed with water, and recrystallized from methanol: dichloromethane mixture to give a white product. Yield: 2.52 g (89%).– Mp 285–287 °C (dec.).– TLC with methylene chloride: methanol (9.2:0.8, v/v).– R_f = 0.56.– IR (cm^{-1})3330 (OH), 3120 (NH), 1665, 1650, 1625 (C = O).– ^{1}H-NMR ([D_6] DMSO) δ 6.82–8.19 (m, 8 H, Ar-H), 9.07 (s, 1H, NH), 10.37 (s, 1H, OH), 12.92 (s,1H, OH); MS, *m/z* (%) 313 (M^+,17), 105 (100,benzoyl cation), 77 (47,phenyl cation). Anal. ($C_{15}H_{11}N_3O_3S$).

*2-(3-Benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**3**)*

To a stirred solution of **2** (3.13 g, 0.01 mol) in dimethylformamide (10 ml), a 10% solution of ferric chloride (25 ml) was added, portionwise. Stirring was continued for 15 min, and the reaction mixture was diluted with water (50 ml), a yellowish-orange precipitate was formed. The precipitate was filtered, washed with water until it is free from the ferric ion, dried, and recrystallized from methanol: dichloromethane mixture to give a yellow solid product. Yield: 1.92 g (95%).– Mp decomposes before melting.– TLC with methylene chloride:methanol (9.4:0.6, v/v).– R_f = 0.52.– IR (cm^{-1}) 3160 (NH), 1665, 1650 (C=O), 1610 (C = N).– ^{1}H-NMR, ([D_6]DMSO) δ6.85–8.10 (m, 8 H, Ar-H and benzoquinone protons), 9.50 (s, 1H, NH); MS, *m/z* (%) 311 (M^+, 100), 282 (3,M^+–CHO), 196 (3,M^+–benzoyl), 149 (6[1,4-benzoquinonyl-CH=N=NH cation]), 105 (30, benzoyl cation), 77 (10, phenyl cation). Anal. ($C_{15}H_9N_3O_3S$).

*2-(4-Phenylpiperazin-1-yl)-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**4a**)*

To a mixture of **3** (0.31 g, 1 mmol) and 4-phenyl piperazine (0.08 g, 0.5 mmol), ethanol (15 ml) followed by 5 drops of glacial acetic acid were added. The mixture was heated under reflux for 0.5 h. The reaction mixture was filtered while hot, and the precipitate was washed with 5 ml of boiling ethanol. The brown solid product was dried, and recrystallized from dichloromethane:ethanol mixture. mp, decomposes before melting;. IR (cm^{-1}) 3120 (NH), 1670 (C=O).– ^{1}H-NMR ([D_6] DMSO) δ 2.96-3.96 (m, 8 H, NCH_2 CH_2N), 6.84–8.12 (m, 12 H Ar-H and quinone protons), 9.32 (s,1H,NH); MS, *m/z* (%) 471 (M^+,13), 366 (14,M^+–benzoyl), 352 (19,M^+–C_6H_5 CONH), 312 (41,M^+–phenylpiperazinyl),291 (10,M^+–[phenylpiperazinyl+ benzamide]), 105 (100, benzoyl cation), 77 (45, phenyl cation).

*2-[4-(N-2-(Thiazolylaminosulfonyl)phenyl)amino]-5-(3-benzoylamino-2,4,5-thiadiazolyl-1,4-benzoquinone (**4b**)*

Obtained from **3** (0.31 g, 1 mmol) and N^1-2-(thiazolyl) sulfanilamide. (0.11 g, 0.5 mmol), as in **4a.** mp, decomposes before melting.– TLC with dichloromethane:methanol (9.2:0.8, v/v).– R_f=0.38.– IR (cm^{-1}) 3400, 3120 (NH); 1665,1640 (C=O); 1320, 1135,1090 (SO). ^{1}H-NMR ([D_6] DMSO) δ6.78–8.25 (m,13 H, Ar H and quinone protons), 12.45 (s, 1H, NH), 12.82 (s, 1H,NH).

*Phenylamino-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**4c**)*

Obtained from **3** (0.31 g, 1 mmol) and aniline (0.047 g, 0.5 mmol), as described for **4a.** mp, decomposes before melting.– TLC with n-hexane:ethyl acetate:methanol (3.0:1.8:0.2, v/v/v) R_f= 0.42.– IR (cm^{-1}) 3240, 3120 (NH), 1665, 1630 (C=O). ^{1}H-NMR ([D_6] DMSO) δ, 6.82–8.16 (m, 12 H, Ar-H and quinone), 9.25 (s,1H,NH), 12.25 (s,1H,NH); MS, *m/z* (%) 402 (M^+,21),400 (100,M^+–2H), 372 (36, M^+ –[2H+CO]), 282 (11,M^+–[C_6H_5CONH]), 105 (8, benzoyl cation), 77 (18,phenyl cation).

*2-(4-Methylphenylamino)-5-(3-benzoylamino-2,4-5-thiadiazolyl)-1,4-benzoquinone (**4d**)*

Obtained from **3** (0.31 g, 1 mmol) and methylaniline (0.054 g, 0.5 mmol), see **4a.** mp, decomposes before melting.– TLC with n-hexane:ethyl acetate:methanol (3.0:1.8:0.2, v/v/v).– R_f = 0.39.– IR (cm^{-1}) 3280, 3120 (NH), 1665 and 1630 (C=O).^{1}H-NMR ([D_6] DMSO) δ 3.56 (s, 3H, CH_3), 6.85–8.17 (m, 11 H, ArH and quinone), 9.05 (s, 1H, NH), 12.65 (s, 1H, NH); MS, *m/z* (%) 416 (M^+, 14), 388 (11, M^+–CO), 105 (100, benzoyl cation),77 (33, phenyl cation).

*2-(4-Hydroxyphenylamino)-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**4e**)*

Obtained from **3** (0.31 g, 1 mmol) and 4-aminophenol (0.055 g, 0.5 mmol), cf. **4a**. mp, decomposes before melting.– TLC with dichloromethane:methanol (9.4:0.6, v/v).– R_f =0.36.– IR (cm^1)3390 (OH), 3180 (NH), 1665–1620 (C = O, C = N).– ^{1}H-NMR ([D_6] DMSO) δ 6.67–8.09 (m, 11 H, Ar-H and quinone protons) 9.53 (s, 1H, NH), 12.56 (s, 1H, NH) 12.89 (s, 1H, NH); MS, *m/z* (%). 418 (M^+, 4), 105 (100, benzoyl cation), 77 (34, phenyl cation).

*2-(4-Bromophenylamino)-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**4f**)*

Obtained from **3** (0.31 g, 1mmol) and 4-bromoaniline (0.086 g, 0.5 mmol), cf. **4a**. mp, decomposes before melting.– TLC with n-hexane:ethyl acetate:methanol (3.0:1.8:0.2, v/v/v).– R_f = 0.40.– IR (cm^{-1}) 3390 (NH); 1660–1625 (C=O, C=N).– ^{1}H-NMR ([D_6] DMSO) δ 6.73–8.21 (m,11 H, Ar-H and quinone protons) 9.15 (s, 1H, NH), 12.85 (s, 1H,NH); MS, *m/z* (%) 481 (M^++2, 59), 479 (46, M^+), 400 (15,M^+ –Br), 105 (100, benzoyl cation), 77 (89, phenyl cation).

*2-(4-Nitrophenylamino)-5-(3-benzoylamino-2,4,5-thiadiazolyl)-1,4-benzoquinone (**4g**)*

Obtained from **3** (0.31g, 1 mmol) and 4-nitroaniline (0.069 g, 0.5 mmol) cf. **4a**. mp, decomposes before melting.– TLC with n-hexane:ethyl acetate:methanol (3.0:1.8:0.2, v/v/v).– R_f = 0.33.– IR (cm^{-1}) 3395 (NH); 1665,

1650 (C = O).– ^{1}H-NMR ([D_6] DMSO) δ 6.95–8.11 (m,11 H, Ar-H and quinone protons), 12.25 (s, 1H, NH), 12.91 (s, 1H,NH); MS, *m/z* (%) 447 (M^+, 9), 419 (46,M^+–CO), 105 (100,benzoyl cation), 77 (85, phenyl cation).

Brine Shrimp Bioassay (BS Test)

This bioassay was performed as described by Meyer *et al* [15]. The samples were prepared by dissolving 10 mg of the compound in a 1:1 ethanol-dimethyl sulfoxide mixture (2 ml). 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). Appropriate amounts from the compounds solution were transferred to vials and dried. A disposable pipette was used to transfer ten shrimps to each sample vial, and artificial sea water was added to make the volume up to 5 ml. The vials were maintained under illumination. The nauplii were counted visually after 24 h. The Probit Analysis Method described by Finney [16] was used to determine the $LC_{50's}$ and 95% confidence intervals.

Cytotoxicity Tests

The cytotoxicity tests were determined in the Purdue Cell Culture Laboratory (Purdue Cancer Center, Purdue University, West Lafayette, IN, USA) against three human cell lines: A-549 (Human Lung Carcinoma); MCF-7 (Human Breast Carcinoma); HT-29 (Human Colon Adenocarcinoma) following protocols established by the National Cancer Institute (NCI) of the USA.

Antimicrobial Activity

Antimicrobial 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 [17] in dextrose broth. 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. N^1-2-(Thiazolyl)sulfanilamide (sulfathiazole) was purchased from Merck Co., (Schuchardt, Germany).

Antifungal activities of the compounds were tested against yeast-like fungi (*Candida albicans* IGR 66-hospital isolate, Institute Gustave-Roussy, France). 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, then diluted in dextrose broth to give an initial concentration of 250 μg/ml. Serial 2-fold dilutions in dextrose broth were made until a final concentration of 0.4 μg/ml is reached. The microorganisms were grown overnight in dextrose broth at 35 °C and diluted to 10^{-3} just before being used. Four control tubes were used: one with dextrose broth only, one with dextrose broth and the test organism, one with the highest drug concentration, and one with the lowest drug concentration. Test tubes containing 2 ml dextrose brtoh were inoculated with 0.05 ml of the diluted overnight test organisms. All tubes were then incubated at 35 °C for 18 h. The lowest concentration at which there was no growth was considered as the minimum inhibitory concentration (MIC).

Mutagenicity Test

The bacterial strains; *Salmonella typhimurium* strains TA 1530, TA 100, TA 1537 and TA 97A were kindly supplied by Prof. B. N. Ames (Department of Biochemistry, University of California, Berekely, USA). Vogel-Bonner medium E (50×), histidine-biotin solution (0.5 M) top agar, minimal glucose plates, histidine-biotin plates and ampicillin plates were prepared as described by Maron and Ames [18].

Stock solution of samples were prepared by dissolving 10 mg of the compound in 1 ml dimethylsulfoxide-ethanol mixture (1:1, v/v), and serial dilutions ranging from 5 mg/ml-to-0.01 mg/ml were prepared.

The plate incorporation test was followed [18]. The top agar was distributed into capped culture tubes which were held at 45 °C in a water bath. To each tube, 0.1 ml of a fresh overnight culture of the tester strain was added, followed by the addition of 0.1 ml of the test compound. Positive and negative controls were used in each assay. The test components were mixed by vortexing the tube for about 3 s at low speed and directly poured onto a minimal glucose agar plates. After 45 min the plates were inverted and placed in a dark 37 °C incubutor. The revertant colonies on the treated as well as on the control plates were counted.

References

[1] R. Finch, *Medicine* **1996**, *1*, 27–30.

[2] T. J. Kress, S. M. Costantino, *J. Heter. Chem.* **1980**, *17*, 607–608.

[3] A M. Osman, K. M. Hassan, H. S. El-Kashef, M. A. El-Maghraby, M. A. Hassan, *J. Indian Chem. Soc.* **1979**, *56*, 521–524.

[4] I. Chaaban, O. O. Oji, *J. Indian Chem. Soc.* **1984**, *61*, 523–525.

[5] A. K. Shakya, R. K. Agrawal, P. Mishra, *J. Indian Chem. Soc.* **1991**, *68*, 147–148.

[6] M. G. Mamolo, L. Vio, E. Banfi, *Farmaco* **1996**, *51*, 71–74.

[7] F. Techou, A. H. Khan, J. S. Driscoll, *J. Med. Chem.* **1976**, *19*, 1302–1308.

[8] M. S. R. Nair, M. Anchel, *Tetrahedron Lett.* **1972**, *9*, 795–796.

[9] R. C. Fuson, *Chem. Rev.* **1935**, *16*, 1–27.

[10] I. M. Roushdi, A. A. Mikhail, I. C. Ahmed, Acta. Pharm. Jugoslav. **1976**, *26*, 287–294.

[11] M. A. Hassan, M. T. Youssef, A. S. Alkofahi, H. D. Tabba, I. M. Labouta, *Acta. Pharmaceutica Turcica*, in press.

[12] I. M. Roushdi, A. A. Mikhail, I. Chaaban, *Acta Pharm. Jugoslav.* **1976**, *26*, 277–285.

[13] I. Chaaban, E. Mohamad, El-Khawass,O. M. Aboulwafa, A. Hazzaa, *Acta. Pharm. Jugoslav.* **1989**, *39*, 143–149.

[14] M. Suzuki, T. Iwasaki, M. Miyoshi, K. Okumura, K. Matsumoto, *j. Org. Chem.* **1973**, *38*, 3571–3575.

[15] B. N.Meyer, N. R. Ferrigni, J. E. Putnam, et al., *Planta Medica* **1982**, *45*, 31–34.

[16] D. J. Finney: *Probit Analysic*, 3rd edn., Cambridge University Press, Cambridge, **1971**.

[17] J. A. Washington, A.L Al-Barry: *Dilution Test Procedures*. In: E. H. Lennette, E. H. Spaulding, J. P. Traunt (Eds), *Manual of Clinical Microbiology*, 2nd edn, PP 410-417. American Society for Microbiology, Washington, **1978**, pp. 410–417.

[18] D. M. Maron, B. N. Ames, *Mut. Res.* **1983**, *113*, 173–215.

[19] E. Hoggarth, *J. Chem. Soc.* **1949**, 1160–1163.

[20] M. Z. Barakat, S. K. Shehab, M. M. El-Sadr, *J. Chem. Soc.* **1958**, 901–902.

[21] T. Zinche, D. V. Hagen, *Chem. Ber.* **1883**, *18*, 786–788.

[22] F. I. Carrol, K. H. Dudley, H. W. Miller, *J. Med. Chem.* **1969**, *12*, 187–189.

[23] M. Suffness, J. Douros, *Cancer Drug Development, Part A*. In: Methods in Cancer Research. V. T. T. Devita, H. Busc (Eds). Vol XVI, Academic Press, New York **1979,** pp 73–123.

[24] G. L. Mandell, M. A. Sande: *Antimicrobial Agents*; S. C. Harvey *Antiseptics, Disinfectants, Fungicides, and Ectoparasiticides*. In: A. G. Gilman, L. S. Goodman, A.Gilman (Eds), *The Pharmacological Basis of Therapeutics*, 6th ed., Macmillan Publishing Co., Inc New York, 1980, pp. 982–1121.

[25] J. McCann, B. N. Ames, *Proc. Natl. Acad. Sci.* **1976**, *73*, 950–954.

Received: July 23, 1998 [FP322]