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Design, Synthesis, and Antimicrobial Activity, of New 1,4-disubstituted Octahydroquinoxaline-2,3-dione Derivatives

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A series of 1,4-disubstituted octahydroquinoxaline-2,3-dione derivatives was prepared through two steps reaction. The latter involves the formation of *N,N*-disubstituted cyclohexane-1,2-diamine derivatives (**la-j**) through reductive alkylation of 1,2-cyclohexanediamine with different aldehydes in presence of sodium cyanoborohydride. Fusion of compounds (**1a-j**) with diethyl oxalate affording the target compounds (**2a-j**). Elucidation of structures of compounds (**2a-j**) was based upon different spectral data as well as the elemental methods of analyses. In addition, mass spectrometry and X-ray diffraction analyses were carried out. Moreover, the lipophilicity of the target compounds as expressed from the Clog P. Most of the test compounds (**2a-j**) showed weak to moderate antibacterial and antifungal activities against most of the used bacterial and fungal strains in comparison to chloramphenicol and clotrimazole as reference drugs respectively.

Key Words : Antimicrobial, Activity, Octahydroquinoxaline-2,3-dione, Diffraction, Synthesis

Introduction

The emergence of microbial resistance is an evolutionary process based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal. Survival of bacteria often results from an inheritable resistance.[1,2] Moreover, antibiotic resistance may impose a biological cost and consequently, spread of antibiotic resistant bacteria may be hampered by reduced fitness associated with the resistance. However, additional mutations may compensate for this fitness cost and aids the survival of this bacteria.[3-6] Hence, the search for new and potent antimicrobial agents is gaining interest. On the other hand, nitrogen containing heterocycles are indispensable structural units for medicinal chemists. Among the various heterocyclic compounds, quinoxalines form an attractive biologically active molecules as those form part of various antibiotics such as echinomycin, levomycin and actinoleutin[7] that are known to possess other biological potentials such as adenosine receptor antagonist, anticancer, anthelimintic, antidepressant, anti-inflammatory, and antituberular activities.[8-13] Moreover, quinoxaline and its analogues have been investigated as the catalyst's ligands.[14] In view of the literature regarding antimicrobial potency of quinoxalines and their mode of action that prevent DNA-directed RNA synthesis by virtue of binding to CpG site on DNA, the quinoxaline nucleus is focused on synthesizing newer derivatives to explore potent antimicrobial activities.[4,8]

The continued interest in designing new flexible quinoxaline and quinoxalinedione molecules stems mainly because of the outstanding biological activities exhibited by several derivatives incorporating such heterocyclic moieties.[15-17] Accordingly, the present work aims to the design and synthesis of new octahydroquinoxalinedione derivatives and elucidation of their structures, in addition to, testing the target compounds for their expected antimicrobial effects, if any.

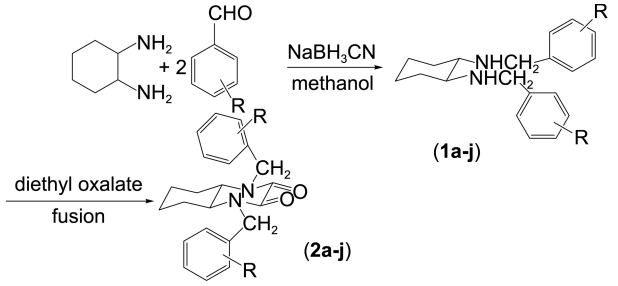
Experimental

Chemistry. Melting points were determined in an electrothermal melting point apparatus (Stuart Scientific, Staffordshine, STIS, SMP3, UK) and were uncorrected. Monitoring of the chemical reactions was carried out by TLC using precoated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Germany) and $CHCl_3/CH_3OH$ as the mobile phase. Visualization of the spots was effected by ultraviolet lamp (model CM-10, USA) at wavelengths 254 and 365 nm and/or iodine stain.

IR spectra were carried out as KBr discs on a Shimadzu IR-470 Spectrometer (Shimadzu, Japan). ^{1}H-NMR spectra were scanned on a Varian EM-360L NMR spectrometer (60 MHz, Varian, USA). Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard; using $CDCl_3$ as a solvent and D_2O was used for the detection of the exchangeable protons.

Electron Impact mass spectra (EI-MS) were run with JEOL JMS600 mass spectrometer at 70 eV (Thermo Electron Corporation, Japan) at the Micro Analytical Central Lab, Assiut University. Elemental microanalyses were performed on a Perkin-Elmer, 240 Elemental Analyzer, at the Unit of Microanalysis, Assiut University and the Micro Analytical Center, Faculty of Science, Cairo University. X-ray diffraction (XRD) was carried out using XRD unit, at Dept., of physics, Faculty of Science, Assiut University.

***N,N*-Disubstituted Cyclohexane-1,2-diamine Derivatives (1a-j).** Sodium cyanoborohydride (48.0 mmol) was added portion wise to a solution of the appropriate aldehyde



R = (**a**) H, (**b**) *p*-Br, (**c**) *p*-Cl, (**d**) *o*-Cl, (**e**) *p*-CH_3, (**f**) *p*-C_3H_7, (**g**) *p*-OCH_3, (**h**) NMe_2, (**i**) *p*-F, and (**j**) *o*,*m*-OCH_2O

Scheme 1. Synthesis of compounds **1a-j** and **2a-j**.

derivative (60 mmol) and 1,2-cyclohexylamine (30 mmol) in methanol (70 mL) at 0 ºC. The reaction mixture was stirred for 3 h at ambient temperature, an additional amount of sodium cyanoborohydride (8.1 mmol) was added portion wise and stirring was continued for further 2 h. The reaction mixture was quenched with distilled water, concentrated and extracted with chloroform; the combined organic extract was washed with distilled water, and dried. Concentration and column chromatography ($CHCl_3/CH_3OH$) afforded compounds (**1a-j**) as pale yellow oils, Scheme 1. Yields and ^{1}HNMR data are given in Table 1.

1,4-Disubstituted Octahydroquinoxaline-2,3-dione (2a-j). A mixture of the *N,N*-disubstituted cyclohexane-1,2-diamine derivatives (**1a-j**) (7.5 mmol), and freshly distilled diethyl oxalate (7.5 mmol) was fussed for 2 h, treated with ether (30 mL) and left overnight. The product was filtered, dried and crystallized from absolute ethanol, Scheme 1, Tables 2, and 3.

Calculation of the log P Values. The log P values of the target compounds (**2a-j**), were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (McLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens were omitted or 'suppressed' (SMILES notation) was entered into the program, which computes the log P based on Crippen's fragmentation[18], and the results are given in Table 2.

X-ray Diffraction. X-ray diffraction (XRD) was carried out using XRD unit, model PW1710 control unit, PW1710 generator and PW1050 Goniometer, Anode material Cu, Optics automatic divergence slit 0.1 and beta filtering graphite monochromator and software visualx, traces and PDF2 1999[19] at Dept., of physics, Faculty of Science, Assiut University.

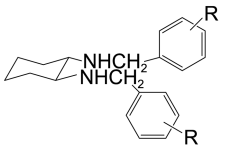
Antimicrobial Activity.

Antibacterial Activity: The synthesized compounds (**2a-j**) were tested for their antibacterial activity *in vitro*, in comparison with chloramphenicol as a reference drug using the standard agar cup diffusion method[20] using six bacterial species representing both Gram-positive and Gram-negative strains at the Assiut University Mycological Center (AUMC). The strains are common contaminants of the environment in Egypt and some of which are involved in human and animal diseases. The used bacterial strains are *Serratia marscens* (AUMC B55), *Pseudomonas aeruginosa* (AUMC B73), and *Escherichia coli* (AUMC B53) as representatives for the Gram negative strains, while the Gram positive strains were represented by *Staphylococcus aureus* (AUMC B54), *Bacillus cereus* (AUMC B52), and *Micrococcus luteus* (AUMC B112).[21,22]

Bacterial strains were individually cultured for 48 h in 100 mL conical flasks containing 30 mL Nutrient Agar (NA) medium. Bioassay was done in 10 cm sterile plastic Petri dishes in which One mL suspension and 15 mL of NA were poured. Plates were shaken gently to homogenize the inocula.

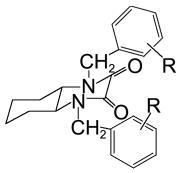
After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork

Table 1. Yields and ^{1}HNMR data of compounds (**1a-j**)



Compd. No.	R	Yield %	^{1}H NMR
1a	H	85	1.00-2.40 (m, 12H, *c*-hexyl, and 2NH), 3.30-3.90 (dd, 4H, $2CH_2$), and 7.20 (s, 10H, 2 Ph).
1b	*p*-Br	95	0.80-2.20 (m, 12H, *c*-hexyl, and 2NH), 3.30-4.00 (dd, 4H, $2CH_2$), and 6.90-7.5 (dd, 8H, 2 Ph).
1c	*p*-Cl	93	0.85-2.25 (m, 12H, *c*-hexyl, and 2NH), 3.33-3.90 (m, 4H, $2CH_2$), and 7.15 (s, 8 H, 2 Ph).
1d	*o*-Cl	67	0.85-2.40 (m, 12 H, *c*-hexyl, and 2NH), 3.15-4.10 (m, 4H, $2CH_2$), and 6.70-7.40 (m, 8 H, 2 Ph).
1e	*p*-CH_3	85	0.85-2.20 (m, 12H, *c*-hexyl, and 2NH), 2.33 (s, 6H, $2CH_3$), 3.30-4.00 (m, 4H, $2CH_2$), and 7.15 (s, 8H, 2Ph).
1f	*p*-*i*-C_3H_7	86	0.85-2.10 (m, 24H, *c*-hexyl, $4CH_3$, and 2NH), 2.50-3.00 (m, 2H, 2CH), 3.35-400 (m, 4H, $2CH_2$), and 7.10 (s, 8H, 2Ph).
1g	*p*-OCH_3	90	0.70-2.30 (m, 12H, *c*-hexyl, and 2NH), 3.30-3.90 (m, 10H, $2CH_2$, and $2CH_3$), and 6.80-7.30 (m, 8 H, 2Ph).
1h	*p*-$N(CH_3)_2$	92	0.85-2.35 (m, 12H, *c*-hexyl, and 2NH), 2.85 (s, 12, $4CH_3$), 3.25-3.85 (m, 4H, $2CH_2$), and 6.40-7.20 (m, 8H, 2Ph).
1i	*p*-F	90	0.85-2.30 (m, 12H, *c*-hexyl, and 2NH), 3.30-4.00 (m, 4H, $2CH_2$), and 6.60-7.33 (m, 8H, 2Ph)
1j	*o*,*m*-OCH_2O	90	0.85-2.33 (m, 12H, *c*-hexyl, and 2NH), 3.20-3.85 (m, 4H, $2CH_2$), 5.85 (s, 4H, $2CH_2$ of pripeonyl), and 6.50-6.85 (m, 6H, 2Ph).

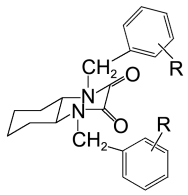
Table 2. The physicochemical data and elemental microanalyses of compounds **2a-j**



Compd No.	R_1	M. formula (M. Wt)	Yield[a] %	mp °C	R_f[b]	ClogP	CHN Found/Cacd		
2a	H	$C_{22}H_{24}N_2O_2$ (348.44)	82	100-2	0.42	5.57	75.57 75.83	7.26 6.94	7.86 8.04
2b	*p*-Br	$C_{22}H_{22}Br_2N_2O_2$ (506.23)	90	170	0.41	7.3	52.09 52.20	4.02 4.38	5.86 5.53
2c	*p*-Cl	$C_{22}H_{22}Cl_2N_2O_2$ (417.33)	90	173	0.52	7.0	63.77 63.32	5.36 5.31	6.61 6.71
2d	*o*-Cl	$C_{22}H_{22}Cl_2N_2O_2$ (417.33)	87	170	0.58	7.0	63.00 63.32	5.54 5.31	6.51 6.71
2e	*p*-CH_3	$C_{24}H_{28}N_2O_2$ (376.49)	75	152	0.32	6.57	76.03 76.56	7.77 7.50	7.19 7.44
2f	*p*-*i*-C_3H_7	$C_{28}H_{36}N_2O_2$ (432.60)	80	150	0.52	8.42	77.35 77.74	8.74 8.39	6.91 6.48
2g	*p*-OCH_3	$C_{24}H_{28}N_2O_4$ (408.49)	85	158	0.35	5.41	70.36 70.57	7.19 6.91	6.63 6.86
2g	*p*-$N(CH_3)_2$	$C_{24}H_{28}N_4O_2$ (404.50)	85	158	0.35	5.90	71.03 71.26	7.18 6.89	13.96 13.85
2i	*p*-F	$C_{22}H_{22}F_2N_2O_2$ (384.42)	85	158	0.35	5.86	68.47 68.74	5.45 5.77	6.94 7.29
2j	*o*,*m*-OCH_2O	$C_{26}H_{24}N_2O_6$ (436.46)	85	158	0.35	5.50	66.04 66.20	5.79 5.53	6.02 6.48

[a]The crystallization solvent for the solid compounds is ethanol. [b]Developing solvent system is $CHCl_3/CH_3OH$ (5:3).

Table 3. ^{1}H NMR and mass fragmentation data of compounds (**2a-j**)



Compd. No.	R	^{1}H NMR	MS
2a	H	1.00-2.90 (m, 8 H, *c*-hexyl), 3.10-3.30 (m, 2H, 2CH of *c*-hexyl), 4.10-4.30 and 5.10-5.30 (dd, 4H, $2CH_2$), and 7.30 (s, 10H, 2 Ph).	M^+-1(347.47, 0.3%) and C_6H_{10}+1 (85, 100%).
2b	*p*-Br	0.75-2.30 (m, 8 H, *c*-hexyl), 3.10-3.60 (m, 2H, 2CH of *c*-hexyl), 4.00-4.50 and 4.70-5.15 (dd, 4H, $2CH_2$), and 6.85-7.50 (m, 8H, 2 Ph).	M^+ (505.81, 0.2%), M^++2 (507.81, 0.2%), M^++4 (509.81, 0.2%), and C_6H_{10}+2 (86, 100%).
2c	*p*-Cl	0.85-2.10 (m, 8 H, *c*-hexyl), 3.00-3.50 (m, 2H, 2CH of *c*-hexyl), 4.10-4.50 and 4.70-5.20 (dd, 4H, $2CH_2$), and 6.70-7.33 (m, 8H, 2 Ph).	M^+ (417.14, 2.7%), M^++2 (419.09, 0.8%), M^+-2Cl (347.14, 0.2%), and $C_7H_5Cl^+$ (124, 100%).
2d	*o*-Cl	0.90-2.10 (m, 8 H, *c*-hexyl), 3.10-3.50 (m, 2H, 2CH of *c*-hexyl), 4.10-4.50 and 4.85-5.20 (dd, 4H, $2CH_2$), and 6.85-7.40 (m, 8H, 2 Ph).	M^+ (417.77, 4.0%), M^++2 (419.10, 1.4%), and $C_7H_5Cl^+$ (124, 100%).
2e	*p*-CH_3	0.85-2.15 (m, 8 H, *c*-hexyl), 2.3 (s, 6H, 2 CH_3), 3.15-3.60 (m, 2H, 2CH of *c*-hexyl), 4.00-4.50 and 4.90-5.15 (dd, 4H, $2CH_2$), and 7.15 (s, 8H, 2 Ph).	M^+ (376.35, 3.8%), M^++1-2CH_3 (347.09, 16.3%), M^++1-2CH_3 (347.09, 16.3%).

Table 3. Continued

Compd. No.	R	^{1}H NMR	MS
2f	*p*-*i*-C_3H_7	0.60-2.20 (m, 20H, *c*-hexyl and 4 CH_3), 2.50-3.00 (m, 2H, 2CH), 3.10-3.50 (m, 2H, 2CH of *c*-hexyl), 4.00-4.50 and 4.80-5.20 (dd, 4H, $2CH_2$), and 7.00- (s, 8 H, 2 Ph).	M^+-*i*-*Pr* (376.35, 3.8%), M^++1-2CH_3 (347.09, 16.3%), M^++1-2CH_3 (347.09, 16.3%).
2g	*p*-OCH_3	0.75-2.15 (m, 8H, *c*-hexyl), 3.00-3.20 (m, 2H, 2CH of *c*-hexyl), 3.70 (s, 6H, 2 CH3), 4.00-4.50 and 4.80-5.30 (m, 4H, $2CH_2$), and 6.50-7.30 (m, 8H, 2 Ph).	M^++1 (409.66, 7.0%), M^+-1 (407.99, 0.3%), *p*-pethoxybenzyl (121.00, 100%).
2h	*p*-$N(CH_3)_2$	0.85-2.10 (m, 8H, *c*-hexyl), 2.95 (s, 12, $4CH_3$), 3.20-3.50 (m, 2H, 2CH of *c*-hexyl), 4.10-4.50 and 5.70-5.40 (m, 4H, $2CH_2$), and 6.33-7.30 (m, 8H, 2 Ph).	M^++1-NMe_2 (391.18, 1.2%), $C_9H_{12}N^+$ (134.03, 100%).
2i	*p*-F	0.80-2.20 (m, 8 H, *c*-hexyl), 3.10-3.60 (m, 2H, 2CH of *c*-hexyl), 4.20-5.20 (m, 4H, $2CH_2$), and 6.85-7.15 (m, 8H, 2 Ph-*p*-F).	M^+ (384.05, 8.0%), M^+-2 (382.85, 45.6%), $C_7H_4F^+$ (105.83, 100%).
2j	*o*,m-OCH_2O	0.90-2.33 (m, 8H, *c*-hexyl,), 3.15-3.70 (m, 2H, 2CH of *c*-hexyl), 4.10-4.50 and 4.85-5.30 (m, 4H, $2CH_2$), 6.00 (s, 4H, 2CH2 of piperonyl), and 6.70 (m, 6H, 2 Ph).	M^++1 (437.07, 1.5%), $C_9H_9O^+$+1 (134.96, 100%).

borer. The test compounds (**2a-j**) and chloramphenicol were dissolved in dimethyl sulphoxide (10 μmol/mL) were loaded in the cavities. In addition, other cavities were loaded with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28 ± 2 ºC for 48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are cited in Table 4.

Antifungal Activity: Compounds (**2a-j**) were tested for their antifungal activity *in vitro*, in comparison with clotrimazole as a reference drug using the standard agar cup diffusion method[20] at the Assiut University Mycological Center (AUMC), Faculty of Science, Assiut University. Six pathogenic [*T. rubrum* (Castellani) Sabouraud AUMC 1145, and *C. albicans* (Robin) Berkhout AUMC 421], phytopathogenic (*F. oxysporum* Schlechtendal AUMC 208) and food deteriorating fungal species [*A. flavus* Link AUMC 3372, *G. candidum* Link AUMC 228, and *S. brevicaulis* (Saccardo) Bainier AUMC 363] were used in the present study.[23-28]

Spore suspension in sterile distilled water was prepared from 7 days old culture of the test fungi growing on Sabouraud' dextrose broth (30 mL) media in 100 mL conical flasks. The final spore concentration was 5×10^4 spores/mL. About 15 mL of growth medium was introduced on sterilized Petri dishes of 10 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. Antifungal activity of the test compounds (**2a-j**) was performed by the standard agar cup diffusion method as follow:

After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer and was filled with the solutions of the test compounds and cotrimazole (10 μmol/mL in DMSO). In addition, other cavities were impregnated with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28 ± 2 ºC for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are cited in Table 5.

The Minimum Inhibitory Concentrations (MICs). The test compounds giving positive results were diluted with DMSO to prepare a series of descending concentration down to 0.15 μmol/mL. Diluted solutions were similarly assayed as mentioned before and the least concentration (below which no activity) was recorded. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog, and the results are cited in Tables 6 and 7.

Results and Discussion

Chemistry. Compounds (**1a-j**) were prepared according to reported procedures.[16] Target compounds, 1,4-disubstituted octahydroquinoxaline-2,3-dione derivatives (**2a-j**) were prepared by fussing compounds (**1a-j**) and freshly distilled diethyl oxalate. Structures of compounds, (**2a-j**) were confirmed by IR, ^{1}H-NMR, and MS, in addition to elemental method of analyses. IR spectra of compounds (**2a-j**) were characterized by lack of the characteristic bands due to NH functions of the intermediates (**1a-j**) and exhibited bands attributed to C=O stretching function at 1676-1620 cm^{-1}. ^{1}H-NMR spectra of compounds (**1a-j**), Table 1 exhibited a general pattern for the cyclohexyl moiety and 2NH (exchangeable with D_2O) groups which is characterized as a multiplet signal between 0.70 and 2.40 ppm. The benzylic CH_2 protons appeared as a doublet of doublet or multiplet around 3.20 and 4.00 ppm. In addition, the specific function groups appeared in positions that are in accordance with the structures. Compound **1e** showed a singlet signal equivalent to 6 protons at 2.33 ppm corresponding to two *p*-CH_3 groups. Moreover, the aromatic protons showed patterns at positions which are in accordance to the structures of the target compounds (**1a-j**). ^{1}H-NMR spectra of compounds (**2a-j**), Table 3 revealed absence of signals corresponding to the exchangeable protons of the 2NH groups proved the process of cyclization. In addition, the spectra showed a general pattern for the cyclohexyl

Table 4. Antibacterial activity of compounds **2a-j** and chloramphenicol (inhibition zone in mm)

Organisms \ Sample No.	**2a**					**2b**			**2c**					**2d**						**2e**				
	10	5	2.5	1.25	0.6	10	5	2.5	10	5	2.5	1.25	0.6	10	5	2.5	1.25	0.6	0.3	10	5	2.5	1.25	0.6
Serratia marcescens (-ve) AUMC No. B-55	11	0	-	-	-	10	8	0	8	8	0	-	-	10	10	8	0	-	-	12	8	0	-	-
Pseudomonas aeruginosa (-ve) AUMC No. B-73	8	8	0	-	-	0	-	-	0	-	-	-	-	0	-	-	-	-	-	13	10	0	-	-
Escherichia coli (-ve) AUMC No. B-53	17	0	-	-	-	14	0	-	21	15	0	-		12	12	10	0	-	-	19	12	10	0	-
Staphylococcus aureus (+ve) AUMC No. B-54	17	13	10	8	0	13	0	-	19	17	13	8	0	15	15	12	10	0	-	18	14	10	8	0
Bacillus cereus (+ve) AUMC No B-52	18	14	10	8	0	23	14	0	25	16	10	0	-	15	15	14	12	10	0	24	14	10	0	-
Micrococcus luteus AUMC No B-112	23	17	11	0	-	15	8	0	22	18	13	0	-	18	18	17	13	0	-	21	18	13	0	-

Organisms \ Sample No.	**2f**						**2g**						**2h**						**2i**				
	10	5	2.5	1.25	0.6	0.3	10	5	2.5	1.25	0.6	0.3	10	5	2.5	1.25	0.6	0.3	10	5	2.5	1.25	06
Serratia marcescens (-ve) AUMC No. B-55	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	11	8	0	-	-
Pseudomonas aeruginosa (-ve) AUMC No. B-73	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	8	8	0	-	-
Escherichia coli (-ve) AUMC No. B-53	14	14	12	11	0	-	8	0	-	-	-	-	16	12	10	0	-	-	15	12	10	8	0
Staphylococcus aureus (+ve) AUMC No. B-54	15	15	12	10	0	-	8	8	0	-	-	-	16	15	13	11	8	0	16	14	10	8	0
Bacillus cereus (+ve) AUMC NoB-52	22	22	20	16	12	0	14	14	14	12	12	0	21	18	14	13	8	0	16	12	8	0	-
Micrococcus luteus AUMC No B-112	18	18	15	14	0	-	8	0	-	-	-	-	18	18	17	13	0	-	18	17	8	0	-

Organisms \ Sample No.	**2j**					Refer.					
	10	5	2.5	1.25	0.6	10	5	2.5	1.25	0.6	0.3
Serratia marcescens (-ve) AUMC No. B-55	8	0	-	-	-	41	40	38	34	28	26
Pseudomonas aeruginosa (-ve) AUMC No. B-73	13	11	0	-	-	16	14	12	12	10	10
Escherichia coli (-ve) AUMC No. B-53	14	8	0	-	-	30	26	26	20	16	14
Staphylococcus aureus (+ve) AUMC No. B-54	18	11	0	-	-	17	17	15	13	12	12
Bacillus cereus (+ve) AUMC NoB-52	18	14	12	8	0	34	34	32	30	28	25
Micrococcus luteus AUMC No B-112	22	0	-	-	-	20	14	12	0	-	-

Refer. = Chloramphenicol as antibacterial standard. AUMC = Assiut University Mycological Center

moiety as a broad multiplet signal at 0.60 and 2.90 ppm. The spectra of the target compounds (**2a-j**) showed a characteristic multiplet equivalent to 2 protons of the methine protons of the cyclohexyl moiety at 3.00 and 3.60 ppm. Also, they showed doublet of doublet at 4.25 and 5.12 ppm corresponding to the benzylic $2CH_2$ groups. MS of compound (**2a-j**) revealed the molecular ion peaks M^+ corresponding to the molecular weight for compounds **2b**, **2c**, **2d**, **2e**, and **2i** and M^++1 for compounds **2g** and **2j**, M^+-1 for compound **2a**, M^+-*i*-Pr for compound **2f** and M^++1-NMe_2 for compound **2h**.

Lipophilicity. The lipophilicity of the target compounds **2a-j** is expressed in the term of Clog P values (Table 2). The values were computed with a routine method called calculated log P (Clog P) contained in a PC-software package as mentioned under the experimental section[18] The target compounds exhibited high values for Clog P, Table 2. This work devoted to study the effect of the lipophilicity on the antimicrobial activity.

X-ray Diffraction (XRD). The X-ray diffraction (XRD) is based on observing the scattered intensity of an X-ray beam hitting a sample as a function of incident and scattered angle, polarization and wave length or energy. On the other

Table 5. Antifungal activity of compounds **2a-j** and clotrizmazole (inhibition zone in mm)

Organisms \ Sample No.	**2a**					**2b**				**2c**				**2d**						**2e**						
	10	5	2.5	1.25	0.6	10	5	2.5	1.25	10	5	2.5	1.25	10	5	2.5	1.25	0.6	0.3	10	5	2.5	1.25	0.6	0.3	0.15
Candida albicans AUMC No. 418	12	8	8	0	-	8	0	-	-	14	10	0	-	14	14	12	0	-	-	12	12	0	-	-	-	-
Geotrichum candidum AUMC No. 226	14	13	12	10	0	18	12	8	0	19	13	12	0	20	20	16	14	12	0	16	15	14	13	10	8	0
Fusarium oxysporum AUMC No. 5119	13	12	10	0	-	0	-	-	-	17	12	10	0	0	-	-	-	-	-	0	-	-	-	-	-	-
Aspergillus flavus AUMC No. 1276	8	8	0	-	-	0	-	-	-	14	8	0	-	12	12	10	0	-	-	8	0	-	-	-	-	-
Scopulariopsis brevicaulis AUMC No. 729	12	8	0	-	-	12	0	-	-	16	8	0	-	0	-	-	-	-	-	10	10	0	-	-	-	-
Trichophyton rubrum AUMC No. 1804	12	10	8	0	-	18	8	0	-	25	13	0	-	20	20	16	10	0	-	20	10	0	-	-	-	-

Organisms \ Sample No.	**2f**						**2g**		**2h**						**2i**			
	10	5	2.5	1.25	0.6	0.3	10	5	10	5	2.5	1.25	0.6	0.3	10	5	2.5	0.125
Candida albicans AUMC No. 418	23	14	13	10	0	-	0	-	18	13	12	0	-	-	13	8	0	-
Geotrichum candidum AUMC No. 226	24	20	16	14	12	0	12	0	22	20	16	14	10	0	14	0	-	-
Fusarium oxysporum AUMC No. 5119	18	18	14	12	10	0	0	-	15	15	13	12	10	0	12	10	8	0
Aspergillus flavus AUMC No. 1276	14	11	0	-	-	-	0	-	13	10	0	-	-	-	0	-	-	-
Scopulariopsis brevicaulis AUMC No. 729	20	14	10	0	-	-	0	-	20	17	13	12	0	-	10	10	0	-
Trichophyton rubrum AUMC No. 1804	28	22	18	8	0	-	0	-	28	22	18	10	0	-	13	0	-	-

Organisms \ Sample No.	**2j**				Refer.						
	10	5	2.5	1.25	10	5	2.5	1.25	0.6	0.3	0.15
Candida albicans AUMC No. 418	12	0	-	-	30	30	30	26	26	26	20
Geotrichum candidum AUMC No. 226	14	12	12	0	24	22	22	22	22	22	20
Fusarium oxysporum AUMC No. 5119	0	-	-	-	22	22	22	22	18	18	16
Aspergillus flavus AUMC No. 1276	0	-	-	-	27	27	27	25	25	25	25
Scopulariopsis brevicaulis AUMC No. 729	0	-	-	-	26	24	23	23	20	20	17
Trichophyton rubrum AUMC No. 1804	14	0	-	-	35	34	34	34	34	34	34

Refer. = clotrizmazole as antifungal standard. AUMC = Assiut University Mycological Center

hand, to identify unknown substance by comparing diffraction data against a data base maintained by the international centre for diffraction data.[19] The latter contains no XRD data about the new compounds (**2a-j**). The X-ray diffraction spectra for compounds **2b** and **2e** are rich in bands corresponding to C, H, Br, and N.

Antimicrobial Activity.

Antibacterial Activity: The test compounds (**2a-j**) were assayed using the standard agar cup diffusion method[20] at a concentration of 10 μmol/mL and those giving positive results were diluted with DMSO to prepare a series of descending concentrations down to 0.15 μmol/mL and were similarly assayed and the least concentration (below which no activity) was recorded as the MIC.

Results of the antibacterial activity, Table 4, indicated that at a concentration of 10 μmol/mL most of the test compounds were active against most of the used bacterial strains. Compounds **2b**, **2c**, and **2d** were inactive against *P. aeruginosa* and compounds **2f**, **2g**, and **2h** were inactive against *S. Marcescens* and *P. aeruginosa*. In addition the test compounds

Table 6. Antibacterial activity (inhibition zone in mm and MICs given in brackets at 10 μmol/mL) of compounds (**2a-j**) and chloramphenicol

Sample No.	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Micrococcus luteus*
2a	11 (5)	8(2.5)	17(5.0)	17(0.9)	18(0.98)	23(1.75)
2b	10(2.5)	-	14(5.0)	13(5.0)	23(3.32)	15(3.8)
2c	8(2.5)	-	21(2.5)	19(0.8)	25(2.2)	22(1.25)
2d	10(1.25)	-	12(1.25)	15(0.6)	15(0.3)	18(0.6)
2e	12(2.87)	13(2.5)	19(1.96)	18(0.98)	24(2.3)	21(1.25)
2f	-	-	14(0.6)	15(0.6)	22(0.3)	18(0.6)
2g	-	-	8(5.0)	8(2.5)	14(0.3)	8(2.5)
2h	-	-	16(1.25)	8(1.25)	21(0.42)	18(2.5)
2i	11(2.5)	8(2.5)	15(0.65)	16(0.3)	18(1.65)	18(0.5)
2j	8(5.0)	13(2.5)	14(3.57)	18(3.3)	18(0.81)	22(5.0)
Refer.	41(0.04)	16(0.07)	30(0.16)	17(0.04)	34(0.006)	20(1.51)

Table 7. Antifungal activity (inhibition zone in mm and MICs given in brackets at 10 μmol/mL) of test compounds (**2a-j**) and clotrizmazole

Sample No.	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Scopulariopsis brevicaulis*	*Trichophyton rubrum*
2a	12(1.54)	14(0.6)	13(1.25)	8(2.5)	12(2.87)	12(1.25)
2b	8(5.0)	18(2.0)	-	-	12(5.0)	18(4.21)
2c	14(2.5)	19(1.5)	17(1.52)	14(3.57)	16(3.96)	25(3.86)
2d	14(1.25)	20(0.3)	-	12(1.25)	-	20(0.64)
2e	12(2.5)	16(0.15)	-	8(5.0)	10(2.5)	20(3.96)
2f	23(1.0)	24(0.36)	18(0.3)	14(2.5)	20(1.79)	28(0.90)
2g	-	12(5.0)	-	-	-	-
2h	18(1.25)	22(0.32)	15(0.3)	13(2.5)	20(0.6)	28(0.96)
2i	13(3.28)	14(5.0)	12(1.25)	-	10(2.5)	13(5.0)
2j	12(5.0)	14(1.25)	-	-	-	14(5.0)
Refer.	30(0.005)	24(0.012)	22(0.005)	27(0.17)	26(0.005)	35(0.003)

(**2a-j**) showed 19.5-29.5% of the antibacterial activity of chloramphenicol against *S. Marcescens*, 50.0-81.5% against *S. Marcescens*, 40.0-63.5% against *E. coli*, 47.1-106.0% against *S. aureus*, 41.5-70.5% against *B. Cereus*, and 40.0-115.0% against *M. Luteus*. Moreover the variation of the antibacterial activity with concentrations was indicated in Table 4. It was noted that the most sensitive organisms to the test compounds were *S. aureus*, *B. cereus*, *E. coli* and *M. Luteus*. It is noteworthy to mention that, compounds **2a**, **2c**, **2e**, **2i**, and **2j** bearing H, *p*-Cl, *p*-CH_3, *p*-F, and piperonyl moieties respectively gave antibacterial activities up to concentrations of 1.25 μmol/mL. The most active derivatives of the target compounds are **2d**, **2f**, **2g**, and **2h** bearing in their structures *o*-Cl, *p*-*i*Pr, *p*-OCH_3, and *p*-NMe_2 moieties giving antibacterial activities at concentrations of 0.6 μmol/mL.

Antifungal Activity: Results of the antifungal activity, Table 5 revealed that the test compounds showed variable activities against the used fungal strains in comparison to clotrimazole as a reference drug. On the other hand, compound **2b** was inactive against *F. oxysporum*, and *A. flavous*, compound **2d** was inactive against *F. oxysporum*, and *S. brevicaulius*, compound **2e** was inactive against *F. oxysporum*, and compound **2i** was inactive against *A. flavous*. Moreover, compound **2g** was nearly completely inactive against the used fungal strains.

In addition the test compounds (**2a-j**) showed 60.0-100.0% of the antifungal activity of clotrimazole against *C. albicans*, 58.5-100.0% against *G. Candidum*, 59.0-82.5% against *F. oxysporum*, 30.0-52.0% against *A. flavus*, 38.5-91.0% against *S. bervicularis*, and 34.4-80.0% against *T. rubrum*. Moreover the variation of the antifungal activity with concentrations was indicated in Table 6. It was noted that, most of the tested compounds showed effective antifungal activity against the used fungi. Also some test compounds were more active than clotrimazole against *F. oxysporum*. Again, It is noteworthy to mention that, the least active antifungal compound was 2 g (R = OMe) giving activity at 10.0 μmol/mL, while compounds **2b**, **2c**, **2i**, and **2j** bearing *p*-Br, *p*-Cl, *p*-F, and piperonyl moieties respectively gave antifungal activity at 2.5 μmol/mL. Compound **2a** (R = H) showed antifungal activity at 1.25 μmol/mL. On the other hand, compounds **2d**, **2f**, and **2h** bearing in their structures *o*-Cl, *p*-*i*Pr, and *p*-NMe_2 moieties respectively gave antifungal activities at concentrations of 0.3 μmol/mL.

Conclusion

A number of 1,4-disubstituted octahydroquinoxaline-2,3-

dione derivatives were prepared through two steps reaction. This protocol involves the formation of *N*,*N*-disubstituted cyclohexane-1,2-diamine derivatives (**1a-j**) followed by cyclization with diethyl oxalate. The structures of the target compounds, 1,4-disubstituted octahydroquinoxaline-2,3-dione derivatives (**2a-j**) were elucidated depending upon different spectral data as well as the elemental methods of analyses. In addition, mass fragmentation and X-ray diffraction analyses were carried out. Moreover, the lipophilicity of the target compounds as expressed from the Clog P and the measured R_f values were cited. The antimicrobial and MIC activities of compounds (**2a-j**) were investigated. Most of the test compounds showed weak to moderate antibacterial activity in comparison to chloramphenicol as a reference drug. The most active compounds were **2d**, **2f**, **2g**, and **2h** with *o*-Cl, *p*-*i*Pr, *p*-OCH_3, and *p*-NMe_2 moieties giving activities at concentrations 0.6 μmol/mL. Also some test compounds were more active than clotrimazole with regards to their antifugal activities. Compounds **2d**, **2f**, and **2h** with *o*-Cl, *p*-*i*Pr, and *p*-NMe_2 moieties respectively gave antifungal activities at concentrations 0.3 μmol/mL.

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