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Synthesis and antimicrobial evaluation of 3-hydrazinoquinoxaline derivatives and their cyclic analogues

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Abstract

A series of quinoxaline derivatives has been synthesized by reacting 3-hydrazinoquinoxalines **1a,b** with many bifunctional reagents. Reaction of **1a,b** with chloroacetyl chloride and ethyl chloroacetate afforded 1-chloromethyl [1,2,4]triazolo[4,3-a]quinoxalines **2a,b** and dihydro[1,2,4]triazino[4,3-a]quinoxalin-2-ones **3a,b** respectively. Condensation of **1a,b** with ethyl acetoacetate and acetylacetone yielded 2-quinoxalinylhydrazonobutanoates **4a,b** and 2-quinoxalinylhydrazono-2-pentanones **5a,b** respectively. Cyclization of **5a,b** gave 3,5-dimethylpyrazolylquinoxalines **6a,b**. Moreover, reaction of compounds **2a,b** with N-phenyl piperazine derivatives afforded 4-(4-Arylpiperazin-1-yl)-1-[(4-arylpiperazin-1-yl) methyl)]triazoloquinoxalines **7a-e.** The prepared compounds were screened for *in vitro* antibacterial and antifungal activities. None of the tested compounds showed significant activity towards *Pseudomonas aeruginosa.* However, remarkable activities were noticed for compounds **5a** and **5b** against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. Compounds **6a** and **6b** lacked any antimicrobial activities against the tested microorganisms.

Keywords

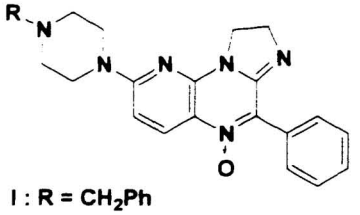
Quinoxalinehydrazones, triazoloquinoxalines, triazinoquinoxalines antimicrobial activity

Introduction

Increasing attention has been paid to the discovery and development of new, more selective antimicrobial agents. Heterocyclic systems having a triazole moiety exhibit a number of interesting biological activities such as antifungal and antibacterial effects [1-4]. A series of 1,2,4-triazolo[4,3-a]quinoxalines has been synthesized and considered as potential candidates for antibacterial activities against both Gram-positive and Gram-negative organisms [5]. Eradex, is a quinoxaline analogue, was reported as fungicidal agent [6]. In addition, Echinomycin [7], Quinaldopeptin [8] and Echinoserine [9] are members of the quinoxaline group antibiotics. Antimicrobial activities have also been shown to be associated with hydrazone derivatives [10]. Furthermore, a literature survey reveals that a variety of antifungal agents contain suitably substituted piperazines such as ketoconazole and compounds of types I and II [11] (Fig.1).

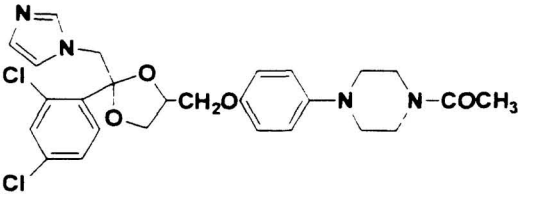
In view of these facts, we decided to design and synthesize certain derivatives of quinoxaline and hydrazinoquinoxaline in addition to triazoloquinoxalines carrying selectively substituted piperazine moieties hoping to obtain highly effective antimicrobial compounds. The present study reports *in vitro* inhibitory activities of the newly prepared compounds against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

Fig. 1.



I : R = CH_2Ph

II : R = CH_3



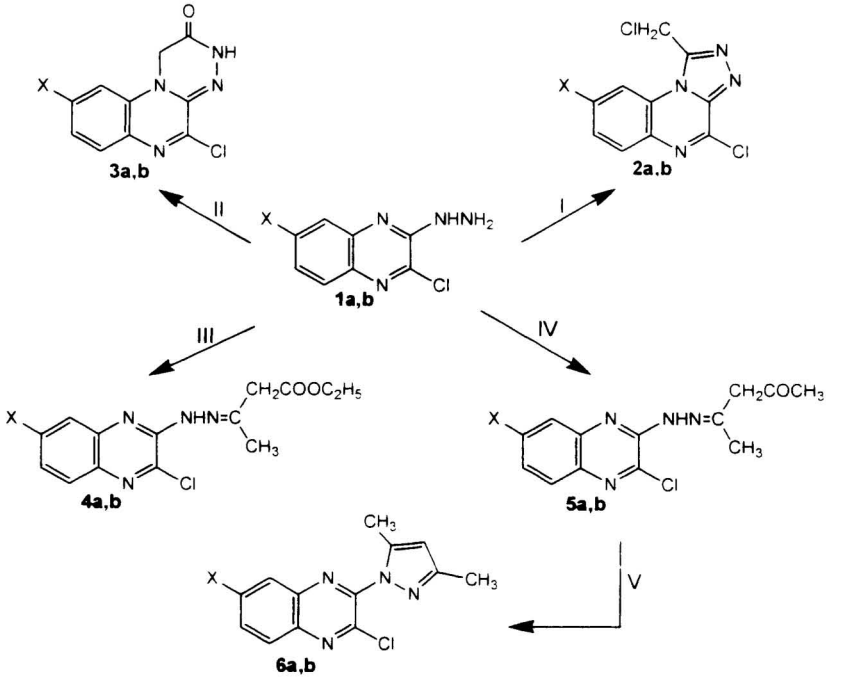
Ketoconazole

Results and Discussion

The preparation of compounds **2-6** has been achieved according to the sequence of reactions (Scheme 1, table 1), in which the hydrazine group in

compounds **1a,b** is subjected to reaction with bifunctional reagents to give the desired compounds. The starting materials, quinoxalinehydrazines **1a** and **1b** were prepared according to the procedure reported by Sarges et al [12], through reaction of ethanolic solution of 2,3-dichloroquinoxaline or 2,3,7-trichloroquinoxaline with two equivalents of hydrazine hydrate. Reaction of **1a,b** with chloroacetyl chloride in refluxing chloroform produced 1-chloromethyltriazolo[4,3-*a*]quinoxaline derivatives **2a,b**. On the other hand, reaction of **1a,b** with ethyl chloroacetate afforded dihydrotriazino[4,3-*a*]quinoxalin-2-ones **3a,b**. The reaction sequence seemed to start with the reaction between the ester and hydrazine under the condition of formation of hydrazide and subsequently ring closure to dihydrotriazinoquinoxalin-2-ones.

Scheme 1

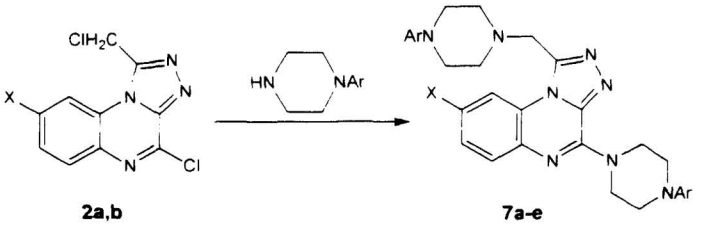


I: $ClCH_2COCl$, **II:** $ClCH_2COOC_2H_5$, **III:** $CH_3COCH_2COOC_2H_5$, **IV:** $CH_3COCH_2COCH_3$, **V:** CH_3COOH

Condensation of 3-hydrazinoquinoxalines **1a,b** with ethyl acetoacetate or acetylacetone at room temperature afforded the corresponding hydrazines **4a,b**

and **5a,b** respectively. In turn, heating **5a,b** with glacial acetic acid resulted in cyclization to compounds **6a,b**. Scheme 2 illustrates the reaction of 4-chloro and 4,8-dichloro-1-chloromethyl[1,2,4]triazolo[4,3-*a*]quinoxaline **2a,b** with *N*-phenylpiperazine derivatives to afford the target compounds **7a-e** (table 1).

Scheme 2



2a,b **7a-e**

Antimicrobial Investigation and Discussion

Antibacterial and antifungal screening was carried out using the agar diffusion technique [13]. The compounds were tested for their activities against the Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*, in addition to pathogenic fungi, *Candida albicans* (table 2). The obtained data revealed that none of the tested compounds showed significant activity towards *Pseudomonas aeruginosa*. Most of the tested compounds showed remarkable activities against *Escherichia coli*. Remarkable activities were noticed for compounds **5a** and **5b** against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. On the other hand, compounds **6a** and **6b** lacked any antimicrobial activities against the tested microorganisms that may indicate that cyclization of the hydrazone moiety of compounds **5a** and **5b** to 2-(1-pyrazolyl)quinoxalines **6a,b** abolished the activity. The most active compounds were **4a,b** and **5a,b** that contain the hydrazone moiety in their structures. This indicates that the hydrazone part in the molecule may share in the antimicrobial activities. A minimum inhibitory concentration (MIC) experiment was performed for the active compounds using the broth dilution technique [14]. Ampicillin, streptomycin and nystatin were used as positive controls. The results are shown in table 3.

Experimental

A. Synthesis

Melting points were recorded on a Fischer-Johns melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian EM-390- (90 MHz) spectrometer using TMS as internal standard (chemical shifts in ppm. δ units). Microanalysis was carried out at the microanalytical unit, Faculty of science, Cairo University, Egypt. The compounds **1a,b** were prepared according to the procedure reported by Sarges *et al* [12]

***4-Chloro- and 4,8-dichloro-1-chloromethyl[1,2,4]triazolo[4,3-a]quinoxaline* 2a,b**

Chloroacetyl chloride (1.13 g, 0.01 mol) was added dropwise to a stirred and well-cooled (ice-bath) suspensions of compound **1** (0.01mol) in chloroform (10 ml). The obtained solution was heated under reflux for 1h, left to cool to room temperature and poured onto ice-cold water. The precipitated product was collected by filtration, dried and crystallized. ^{1}H-NMR (DMSO-d_6), **2a**: 5.72 (s, 2H, CH_2), 7.63-8.43 (m, 4H, Ar-H). **2b**: 5.80 (s, 2H, CH_2), 7.72-8.40 (m, 3H, Ar-H).

***5-Chloro-1,3-dihydro-2H-[1,2,4]-triazino[4,3-a]quinoxalin-2-one* 3a and 5,9-*dichloro-1,3-dihydro-2H-[1,2,4]-triazino[4,3-a]quinoxalin-2-one* 3b**

Ethyl chloroacetate (0.17 g, 1.36 mmol) was added to an ice-cold solution of **1** (1.36 mmol) in chloroform (15 ml). The obtained solution was heated under reflux for 1h, left to cool to room temperature and poured onto crushed ice. The formed precipitate was filtered, washed with water, dried and crystallized). ^{1}H-NMR (DMSO-d_6), **3a**: 4.50 (s, 2H, CH_2), 7.34-8.20 (m, 4H, Ar-H). **3b**: 4.52 (s, 2H,CH_2), 7.34-8.10 (m, 3H, Ar-H)

***Ethyl 2-[(3-Chloro-2-quinoxalinyl)hydrazono]butanoate* 4a and Ethyl 2-[(3,7-*dichloro-2-quinoxalinyl)hydrazono]butanoate* 4b**

A solution of **1** (1.36 mmol) in a mixture of chloroform/methanol ((1:1 v/v) was treated with ethyl acetoacetate (0.18 g 1.38 mmol) and the mixture was allowed

to stir at room temperature for 24 h. The solvent was evaporated under reduced pressure, the residue was extracted with light petroleum ether (40-60), concentrated and left overnight. The product obtained was filtered, dried and crystallized. ^{1}H-NMR (DMSO-d_6). **4a**: 1.23 (t, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.01 (q, 2H, CH_2), 4.14 (s, 2H, CH_2), 7.51-8.10 (m, 4H, Ar-H), 10.83 (br. s, 1H, NH D_2O-exchang.). **4b**: 1.23 (t, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.09 (q, 2H, CH_2), 4.14 (s, 2H, CH_2), 7.52-8.15 (m, 3H, Ar-H), 10.85 (br. s, 1H, NH D_2O-exchang.).

4-[(3-Chloro-2-quinoxalinyl)hydrazono]pentan-2-one **5a and** ***4-[(3,7-dichloro-2-quinoxalinyl)hydrazono]pentan-2-one*** **5b**

A solution of **1** (1.36 mmol) in chloroform/methanol (1:1 v/v) was treated with acetylacetone (0.3 g, 3 mmol) and the mixture was left with stirring at room temperature for 24 h. The solution was concentrated under reduced pressure . The solid product was filtered, dried and crystallized . ^{1}H-NMR (DMSO-d_6). **5a**: 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.95-8.20 (m, 4H, Ar-H) . 10.80 (br s, 1H, NH D_2O-exchang.). **5b**: 2.30 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 7.85-8.40 (m, 3H, Ar-H), 10.12 (br s, 1H, NH D_2O-exchang.).

3-Chloro and 3,7-dichloro-2-(3,5-dimethylpyrazol-1-yl)quinoxalines **6a,b**

A suspension of **5** (0.32 mmol) in glacial acetic acid (2 ml) was heated under reflux for 4 h, concentrated to half volume and left to cool to room temperature. The reaction mixture was diluted with water, filtered, dried and crystallized. ^{1}H-NMR (DMSO-d_6). **6a**: 2.72 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 6.91-7.85 (m, 5H, Ar-H). **6b**: 2.74 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 6.95-7.90 (m, 4H, Ar-H).

4-(4-Arylpiperazin-1-yl)-1-[(4-arylpiperazin-1-yl)methyl)]-[1,2,4]triazolo[4,3-a]quinoxalines **7a,c,d and** ***4-(4-Arylpiperazin-1-yl)-1-[(4-arylpiperazin-1-yl)methyl)]-8-chloro-[1,2,4]triazolo[4,3-a] quinoxalines*** **7b,e**

A mixture of **2** (0.2 mmol), substituted phenylpiperazine (0.6 mmol) and 2 drops of pyridine was heated under reflux in ethanol (10 ml) for 6-8 h. After cooling, the mixture was concentrated under reduced pressure, and poured onto water. The

obtained product was filtered, dried and crystallized . ^{1}H-NMR (DMSO-d_6), **7b**: 2.74-3.50 (m, 16H, piperazine-H), 4.29 (s, 2H, CH_2), 6.76-8.33 (m, 13H, Ar-H). **7c**: 2.69-3.36 (m, 16H, piperazine-H), 3.67(s, 3H, OCH_3), 3.70(s, 3H, OCH_3) 4.28 (s, 2H, CH_2), 6.82-8.36 (m, 12H, Ar-H). **7d**: 2.69-3.36 (m, 16H, piperazine-H), 3.67 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.22 (s, 2H, CH_2), 6.82-8.26 (m, 12H, Ar-H). **7e**: 2.70-3.36 (m, 16H, piperazine-H), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2), 6.85-8.35 (m, 11H, Ar-H).

Table 1. Physicochemical data of compounds 2-7

Comp.	X	Ar	Molecular Formula*	M.p.^{0}C	Cryst.** Solvent	Yield %
2a	H	-	$C_{10}H_6Cl_2N_4$	205-207	E	55
2b	Cl	-	$C_{10}H_5Cl_3N_4$	250-252	E	48
3a	H	-	$C_{10}H_7ClN_4O$	179-181	E	40
3b	Cl	-	$C_{10}H_6Cl_2N_4O$	187-189	E	38
4a	H	-	$C_{14}H_{15}ClN_4O_2$	122-124	D-P	53
4b	Cl	-	$C_{14}H_{14}Cl_2N_4O_2$	170-172	D-P	42
5a	H	-	$C_{13}H_{13}ClN_4O$	120-122	D	68
5b	Cl	-	$C_{13}H_{12}Cl_2N_4O$	110-112	A-E	62
6a	H	-	$C_{13}H_{11}ClN_4$	245-247	A-E	58
6b	Cl	-	$C_{13}H_{10}Cl_2N_4$	>300	A-E	53
7a	H	C_6H_5	$C_{30}H_{32}N_8$	215-217	C-E	67
7b	Cl	C_6H_5	$C_{30}H_{31}ClN_8$	235-237	C-M	54
7c	H	C_6H_4(2-OCH_3)	$C_{32}H_{36}N_8O_2$	195-197	C-E	42
7d	H	C_6H_4(4-OCH_3)	$C_{32}H_{36}N_8O_2$	187-189	C-E	63
7e	Cl	C_6H_4(4-OCH_3)	$C_{32}H_{35}ClN_8O_2$	142-144	C-E	56

*** Satisfactory microanalyses obtained :C,H,N values are within ± 0.4% of theoretical values.** Crystalization Solvents: C) chloroform ; D) DMF ; E) ethanol ; M) methanol; P) petroleum ether (40-60) ; A) water.**

In-vitro antimicrobial screening

Test organisms

Bacteria: *Staphylococcus aureus* ATCC 06538, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536 were cultivated in nutrient agar and nutrient broth. Fungi: *Candida albicans* ATCC 1023 were grown in sabouraud agar and liquid sabouraud.

Table 2: Antimicrobial screening results of the tested compounds:

No.	*E. coli*	*S. aureus*	*C. albicans*
2a	8	-	-
2b	7	-	-
3a	9	-	-
3b	8	-	-
4a	18	8	9
4b	18	8	9
5a	14	14	13
5b	13	18	13
6a	-	-	-
6b	-	-	-
7a	8	-	-
7b	8	-	-
7c	9	9	7
7d	9	8	8
7e	14	7	7
Ampicillin.	0	24	0
Streptomycin	21	20	0
Nystatin.	0	0	21

Pseudomonas aeruginosa records are negative.

Table 3: Minimum inhibitory concentration (μg/ml) of the tested compounds:

No.	E. coli	S. aureus	C. albicans
4a	1.8	12.3	12.3
4b	1.8	12.1	12.1
5a	6.25	6.25	6.25
5b	6.25	1.3	6.25
7a	12	>200	>200
7b	12	>200	>200
7c	12.5	12.5	12.5
7d	12.5	12.3	12.3
7e	6.25	12.3	12.3
Ampicillin.	-	1	-
Streptomycin	3	4	-
Nystatin.	-	-	2

The minimum inhibitory concentration (MIC) is the lowest concentration of compounds that completely inhibits the visible growth of microorganisms.

Evaluation of antimicrobial activity

The tested compounds were first dissolved in dimethyl formamide (DMF) and then diluted with water at the required quantities (0.1 % w/v). In order to ensure that the solvent had no effect on bacteria growth, an inoculated control test was performed with only DMF at the same dilution used in our experiment and found inactive in culture media. A volume of the solution of each of the test compounds equivalent to 100 μg was placed separately in cups, put in the agar. Cultures were incubated for 24 h at 37°C for bacteria and 48 h at 25°C for fungi. Results are recorded as average diameter of inhibition zone in mm (table 2).

Minimum inhibitory concentration (MIC) measurements

The substances dissolved in DMF at 1 mg/ml were diluted in broth in the range 200-10 μg/ml. Inocula were prepared from well-growing overnight cultures of each test organism such that the final inoculum size was ca 10^6 cells/ml. The tubes were then inoculated with 0.1 ml of inoculum and the results are presented as μg/ml and the lowest concentration of compounds that completely inhibits the

visible growth of microorganisms was considered to be the minimum inhibitory concentration (MIC) expressed in μg/ml (table 3). MIC was the mean of three measurements. Ampicillin. streptomycin and nystatin were used as reference antibiotics.

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