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Synthesis of Certain Substituted Quinoxalines as Antimicrobial Agents (Part II)

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Several fused triazolo and ditriazoloquinoxaline derivatives such as 1-aryl-4-chloro-[1,2,4]triazolo[4,3-a]quinoxalines (**3a-d**), 4-alkoxy[1,2,4]triazolo[4,3-a]quinoxalines (**4a,b**), 4-substituted-amino-[1,2,4] triazolo[4,3-a]quinoxalines (**5a-h**), 1-(aryl)-[1,2,4]triazolo[4,3-a]quinoxalin-4(5H)-thione (**6**), 4-(arylidenehydrazino)-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxalines (**10a-e**) and [1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline derivatives (**11-13**) have been synthesized and some of these derivatives were evaluated for antimicrobial and antifungal activity in vitro. It was found that compounds **3a** and **9b** possess potent antibacterial activity compared to the standard tetracycline.

Key words: Ditriazoloquinoxalines, Triazoloquinoxalines, Triazoloquinoxalinethione, Synthesis, Antibacterial agents

INTRODUCTION

Quinoxaline containing compounds exhibit a wide variety of biological activities. It has been reported that some quinoxaline derivatives display antibacterial (Kurasawa *et al.*, 1986; Dirlam *et al.*, 1983), antifungal (Reddy-Sastry *et al.*, 1990; El-Hawash *et al.*, 1999), anti-HIV (Campiani *et al.*, 2001) and anticancer activities (Yoo *et al.*, 1998). Other biological activities exhibited by quinoxaline-containing molecules include antidepressant (Trivedi and Bruns, 1988 , antidiabetic (Reddy-Sastry *et al.*, 1989) and anti-inflammatory activities (Vierfond *et al.*, 1990) The quinoxaline structure is relatively simple and allows for diverse synthetic modifications. Because of this, and because of the importance of quinoxaline as new antibacterials, many reports have appeared describing new analogues (Kurasawa *et al.*, 1986; Dirlam *et al.*, 1983). Meanwhile, the incidence of drug resistance in gram positive bacteria is growing rapidly and has become a significant public health threat. Therefore, we became interested in synthesis of new quinoxaline analogues in an attempt to find an effective antibacterial agent.

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MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were carried out using Perkin-Elmer 240C Microanalyser at the Microanalytical Unit, Cairo University. IR spectra were recorded in KBr using Shimadzu IR 435 Spectrophotometer (ν in cm^{-1}). ^{1}H-NMR spectra were measured on a Jeol NMR FXQ-300 MHz Spectrometer using TMS as an internal standard and DMSO-d_6 as a solvent (chemical shifts in δ, ppm). Thin layer chromatography (TLC) was carried out on percolated plates (silica gel, 60 F-254, Merck) and spots were visualized with iodine or UV light.

2-(Arylidenehydrazino)-3-chloroquinoxalines (2a-d)

General procedure: A mixture of 2-chloro-3-hydrazinoquinoxaline (**1a**) (0.097 g, 0.005 mol), the properly substituted benzaldehyde (0.005 mol) and absolute ethanol (30 mL) was allowed to stir at room temperature for 2 hours. The product formed was filtered, washed with ethanol and crystallized from the appropriate solvent to afford **2a-d** (Table I, II).

1-Aryl-4-chloro-[1,2,4]triazolo[4,3-a]quinoxalines (3a-d)

General procedure: A solution of bromine (0.5 mL, 0.01 mol) in glacial acetic acid (4.5 mL) was added to a sus-

Table I. Physical and analytical data of the prepared compounds

Comp. No.	Yield %	m.p. °C Solvent of cryst.	Formula (Mol. Wt.)	Analysis % Calcd/found C	H	N
2a	88	225-227	$C_{15}H_{10}ClN_5O_2$	54.97	3.08	21.37
		methanol	(327.5)	55.00	3.20	21.00
2b	86	170-172	$C_{15}H_{10}ClN_5O_2$	54.97	3.08	21.37
		methanol	(327.5)	55.30	3.00	21.00
2c	83	149-151	$C_{15}H_{10}ClFN_4$	59.90	3.33	18.64
		CH_2Cl_2	(300.5)	59.60	3.60	19.00
2d	90	164-166	$C_{16}H_{13}ClN_4$	64.76	4.38	18.89
		ethanol	(296.5)	64.70	4.10	18.90
3a	88	>300	$C_{15}H_8ClN_5O_2$	55.30	2.46	21.51
		ethanol	(325.5)	55.30	2.80	21.60
3b	77	228-230	$C_{15}H_8ClN_5O_2$	55.30	2.46	21.51
		2-propanol	(325.5)	55.20	2.60	21.10
3c	86	244-246	$C_{15}H_8ClFN_4$	60.32	2.70	18.76
		gl. acetic acid	(298.5)	60.20	2.50	19.20
3d	82	222-224	$C_{16}H_{11}ClN_4$	65.20	3.76	19.01
		2-propanol	(294.5)	65.30	4.00	19.40
4a	65	193-195	$C_{16}H_{11}N_5O_3$	59.81	3.43	21.81
		CH_2Cl_2	(321)	60.20	3.40	22.00
4b	62	208-210	$C_{17}H_{13}N_5O_3$	60.90	3.88	20.90
		CH_2Cl_2	(335)	61.30	3.70	20.80
5a	72	288-290	$C_{19}H_{16}N_6O_3$	60.64	4.26	22.34
		ethanol	(376)	60.70	4.50	22.10
5b	80	188-190	$C_{20}H_{18}N_6O_2$	64.17	4.81	22.46
		1-butanol	(374)	63.80	4.40	22.50
5c	82	216-218	$C_{19}H_{16}N_6O_2$	63.33	4.44	23.33
		CH_2Cl_2	(360)	62.90	4.10	23.50
5d	84	212-214	$C_{20}H_{19}N_7O_2$	61.70	4.88	25.19
		ethanol	(389)	61.80	4.80	24.80
5e	58	220-222	$C_{17}H_{14}N_6O_2$	61.08	4.19	25.15
		1-butanol	(334)	61.50	4.50	24.90
5f	55	>300	$C_{16}H_{12}N_6O_2$	60.0	3.75	26.25
		ethanol	(320)	59.60	3.80	26.00
5g	74	245-247	$C_{17}H_{15}N_7O_2$	58.45	4.33	28.07
		ethanol	(349)	58.60	4.00	28.30
5h	72	259-261	$C_{21}H_{15}N_7O_2$	63.47	3.80	24.67
		aq. DMF	(397)	63.40	4.00	24.80
6	58	290-291	$C_{15}H_9N_2O_2S$	55.72	2.8	21.66
		gl.acetic acid.	(323)	55.30	2.80	21.60
7	85	276-278	$C_{17}H_{11}N_5O_4S$	53.54	2.91	18.36
		aq. ethanol	(381)	53.60	3.30	18.00
8	88	221-222	$C_{19}H_{15}N_5O_4S$	55.74	3.69	17.11
		ethyl acetate	(409)	55.70	3.80	16.80
9a	65	>300	$C_{15}H_{12}N_6$	65.22	4.35	30.43
		aq DMF	(276)	65.40	4.60	30.80
9b	62	>300	$C_{15}H_{11}N_2O_2$	56.07	3.43	30.53
		aq DMF	(364)	56.30	3.80	30.40
10a	85	272-274	$C_{22}H_{16}N_6$	72.53	4.40	23.08
		DMF	(364)	72.80	4.30	23.00
10b	84	292-294	$C_{22}H_{15}FN_6$	69.11	3.93	21.99
		acetone	(382)	68.90	4.00	22.00
10c	76	287-289	$C_{22}H_{16}N_6O$	69.47	4.21	22.11
		toluene	(380)	69.80	3.80	22.30
10d	81	260-262	$C_{20}H_{14}N_6O$	67.8	3.96	23.73
		toluene	(354)	67.70	4.30	23.60
10e	85	257-259	$C_{24}H_{18}N_6$	73.85	4.62	21.54
		DMF	(390)	73.60	4.50	21.10
11	65	>300	$C_{16}H_9N_7O_2$	58.00	2.74	29.59
		DMF	(331)	57.90	2.80	29.30
12	72	>300	$C_{19}H_{13}N_7O_4$	55.58	3.25	24.31
		CH_2Cl_2	(403)	56.60	3.30	24.40
13	77	>300	$C_{20}H_{15}N_7O_4$	57.55	3.62	23.49
		ethanol	(417)	57.60	3.80	23.10
14	67	236-237	$C_{21}H_{17}N_7O_5$	56.38	3.83	21.91
		CH_2Cl_2	(447)	56.60	4.00	21.60
15	54	>300	$C_{19}H_{13}N_7O_4$	56.58	3.25	24.31
		n-butanol	(403)	56.40	3.50	24.00
16	61	>300	$C_{23}H_{13}N_7O_4$	61.20	2.90	21.72
		ethyl acetate	(451)	61.00	3.30	21.80
17	78	224-226	$C_{20}H_{15}N_7O_2$	62.34	3.92	25.44
		ethanol	(385)	62.20	3.80	25.30

pension of the corresponding hydrazone (**2**) (0.01 mol) and anhydrous sodium acetate (1.65 g, 0.02 mol) in glacial acetic acid (20 mL). The reaction mixture was stirred at room temperature for 1 hour and then poured on to 0.5 N sodium hydroxide solution (100 mL). The separated product was filtered, washed with water, dried and crystallized from the appropriate solvent to yield **3a-d** (Table I, II).

4-Alkoxy-1-(3-nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxalines (4a,b)

General procedure: Compound **3a** (0.65 g, 0.002 mol) was dissolved in ethanol or methanol (10 mL) by gentle heating. The resulting solution was added portionwise to sodium alkoxide solution (prepared by addition of sodium metal granules (0.046 g, 0.002 mol) to the corresponding alcohol (20 mL)). The resulting mixture was stirred at room temperature for 4 hours then kept overnight. The separated solid was filtered, washed with water and crystallized from the appropriate solvent to yield **4a,b** (Table I, II).

1-(3-Nitrophenyl)-4-substituted-amino-[1,2,4]triazolo [4,3-a] quinoxalines (5a-h)

General procedure: A mixture of **3a** (0.65 g, 0.002 mol) and the appropriate amine (0.008 mol) in ethanol (20 mL) was heated under reflux for 8 hours. The mixture was then reduced to half its volume by distillation under diminished pressure and allowed to cool. The formed precipitate was collected by filtration, washed with ethanol and crystallized from the appropriate solvent to afford **5a-h** (Table I, II).

1-(3-Nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4 (5*H*)-thione (6)

A mixture of **3a** (0.65 g, 0.002 mol) and thiourea (0.23 g, 0.003 mol) was heated under reflux in absolute ethanol (20 mL) for 3 hours. The reaction mixture was allowed to cool to room temperature and excess solvent was removed by distillation under diminished pressure. The crude isothiouronium salt was combined with aqueous sodium hydroxide solution (10%, 20 mL) and the mixture was heated under reflux for 2 hours, cooled to room temperature and acidified with glacial acetic acid. The resulting yellow precipitate was collected by filtration, washed with water and dried. The crude product was crystallized to afford **6** as

Table II. Spectral data for prepared compounds

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm), MS m/z (% relative abundance)
2a	**IR:** 3350 (NH), 1615 (C=N).
2b	**IR:** 3350 (NH), 1620 (C=N).
2c	**IR:** 3350 (NH), 1620 (C=N).
2d	**IR:** 3350 (NH), 1617 (C=N). **MS:** 296 (M^+, 13.7%), 297 (M+1, 23.4%), 298 (M+2, 5.4%), 179 (100%).
3a	**IR:** 1620 (C=N) **MS:** 325 (M^+, 21.67%), 326 (M+1, 8.17%), 327 (M+2, 20.44%), 179 (100%).
4a	**IR:**2950 (CH aliphatic), 1620 (C=N)
4b	**IR:** 2950 (CH aliphatic), 1620 (C=N) **MS:** 335 (M^+, 24.9%), 336 (M+1, 5.6%), 320 (100%). **^{1}H-NMR:** 1.62 (t, 3H, CH_3), 4.8 (q, 2H, CH_2), 7.2-8.7 (m, 8H, ArH).
5a	**IR:**1610 (C=N) **MS:** 3.93 (t, 4H, -N(CH_2)$_2$), 4.47 (t, 4H, O(CH_2)$_2$), 6.9-8.7 (m, 8H, ArH).
5e	**IR:** 1616 (C=N) **MS:** 334 (M^+, 13.7%), 335 (M+1, 2.51%), 319 (2.72%), 305 (28.68%), 176 (100%)
6	**IR:** 3350 (NH), 1610 (C=N) **MS:** 323 (M^+, 100%), 324 (M+1, 83%), 325 (M+2, 14.9%).
7	**IR:** 2900-3400 (OH of COOH, broad), 1720 (C=O), 1600 (C=N). **MS:** 381 (M^+, 2.8%), 382 (M+1, 1.7%), 337 (100%). **^{1}H-NMR:** 4.26 (s, 2H, CH_2), 7.3-8.7 (m, 8H), 12.3 (s, 1H, -COOH, exchangeable with D_2O).
8	**IR:** 2950 (CH aliphatic), 1720 (C=O), 1600 (C=N). **MS:** 409 (M^+, 12.3%), 410 (M+1, 12.0%), 411 (M+2, 2.9%), 337 (100%).
9a	**IR:**3300 (NH, NH_2), 1600 (C=N).
9b	**IR:** 3300 (NH, NH_2), 1600 (C=N). **MS:** 321 (M^+, 100%), 322 (M+1, 18.3%), 323 (M+2, 2.37%).
10a	**IR:** 3300 (NH), 1620 (C=N).
10c	**IR:** 3300 (NH), 1620 (C=N). **MS:** 380 (M^+, 6%), 381 (M+1, 56%), 382 (M+2, 52%), 193 (100%).
11	**IR:** 1600-1620 (C=N). **MS:** 332 (M+1, 71.9%), 333 (M+2, 14.3%), 128 (100%).
12	**IR:** 2950 (CH aliphatic), 1725 (C=O), 1620 (C=N). **MS:** 403 (M^+, 26.5%), 404 (M+1, 15.3%), 405 (M+2, 7%), 331 (100%).
13	**IR:** 2950 (CH aliphatic), 1740 (C=O), 1620 (C=N). **^{1}H-NMR:** 1.17 (t, 3H, CH_3), 4.17 (q, 2H, CH_2), 4.8 (s, 2H, CH_2), 7.3-7.7 (m, 4H, ArH), 8.1 (d, 2H), 8.54 (d, 2H).
14	**IR:** 2950 (CH aliphatic), 1730, 1710 (2 C=O), 1610 (C=N) **MS:** 447 (M^+, 1.74%), 448 (M+1, 0.57%), 405 (1.3%), 363 (31.23%), 321 (100%).
15	**IR:** 3400 (NH), 2950 (CH aliphatic), 1730 (C=O), 1620 (C=N). **^{1}H-NMR:** 3.1 (s, 4H, (CH_2)$_2$), 7.3-7.7 (m,4H, ArH), 8.25 (d, 2H), 8.64 (d, 2H), 11.8 (s, 1H, NH, exchangeable with D_2O).
16	**IR:** 3300-3660 (OH, broad, COOH), 1740 (C=O, COOH), 1620 (C=N). **MS:** 451 (M^+, 88.4%), 452 (M+1, 60.9%), 406 (100%), 360 (40.7%). **^{1}H-NMR:** 7.2-8 (m, 8H, ArH), 8.12 (d, 2H), 8.48 (d, 2H), 11.1 (s, 1H, COOH, exchangeable with D_2O). **^{13}C-NMR:**116.6-149.7 (22 C, aromatic and heterocyclic C), 166.2 (1C, COOH).
17	**IR:** 2950 (CH aliphatic), 1620 (C=N) **^{1}H-NMR:** 2.43 (s, 3H, C^3-CH_3), 2.68 (s, 3H, C^5-CH_3), 6.2 (s, 1H, pyrazole C^4-H), 7.26-7.67 (m, 4H, ArH), 7.96 (d, 2H), 8.51 (d, 2H).

yellow crystals (Table I, II).

4-Carboxymethylmercapto-1-(3-nitrophenyl)[1,2,4]-triazolo[4,3-a]quinoxaline (7)

Method A: Compound **6** (0.65 g, 0.002 mol) was dissolved in 10% sodium hydroxide (10 mL) and then a solution of chloroacetic acid (0.19 g, 0.002 mol) in 10% NaOH (5 mL) was added dropwise with stirring. After complete addition, the mixture was refluxed for 3 hours, cooled to room temperature and acidified with 2N hydrochloric acid. The yellow precipitate formed was filtered, washed with water and crystallized to yield **7** as yellow crystals.

Method B: A mixture of **3a** (0.65 g, 0.002 mol),

thioglycolic acid (0.14 mL, 0.002 mol) and triethylamine (1.1 mL, 0.008 mol) in ethanol (20 mL) was refluxed for 5 hours on a steam bath. The solution was then cooled, diluted with water (20 mL) and acidified with 2N hydrochloric acid. The yellow precipitate formed was filtered, washed with water and crystallized to afford **7** (Table I, II).

4-Ethoxycarbonylmethylmercapto-1-(3-nitrophenyl)-[1,2,4]-triazolo[4,3-a]quinoxaline (8)

A mixture of **3a** (0.65 g, 0.002 mol), ethyl mercaptoacetate (0.22 mL, 0.002 mol) and anhydrous potassium carbonate (0.55 g, 0.004 mol) in dimethylformamide (10 mL) was refluxed for 6 hours. After cooling to room temperature, the mixture was diluted with water (30 mL). The precipitated solid was filtered, washed with water and crystallized yield **8** as yellow crystals (Table I, II).

1-Aryl-4-hydrazino-[1,2,4]triazolo[4,3-a]quinoxalines (9a,b)

General procedure: A mixture of **1b** (1.76 g, 0.01 mol) and the corresponding aromatic acid (0.01 mol) in phosphorus oxychloride (20 mL) was refluxed for 3 hours. The excess phosphorus oxychloride was distilled under diminished pressure. Dioxane (25 mL) was added to the residue followed by hydrazine hydrate (4 mL, 0.08 mol) and the mixture was refluxed for 3 hours. The reaction mixture was then cooled to room temperature and the formed precipitate was filtered, washed with ether (20 mL) and crystallized from the appropriate solvent to yield **9a,b** (Table I, II).

4-(Arylidenehydrazino)-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxalines (10a-e)

General procedure: A mixture of **9a** (0.55 g, 0.002 mol) and the corresponding aromatic aldehyde (0.002 mol) was refluxed in ethanol (20 mL) for 1 hour. The solution obtained was concentrated by evaporation to one forth of its original volume under reduced pressure, then allowed to cool in an ice bath. The precipitated solid was filtered, washed with ether and crystallized from the appropriate solvent to afford **10a-e** (Table I, II).

1-(4-Nitrophenyl)-[1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline (11)

A mixture of **9b** (0.96 g, 0.003 mol) and triethyl orthoformate (15 mL) was heated in an oil bath at 150°C with continuous stirring for 10 hours. After cooling to room temperature, the precipitated orange solid was filtered, washed with ethanol and crystallized to afford **11** (Table I, II).

1-Ethoxycarbonyl-6-(4-nitrophenyl)-[1,2,4]ditriazolo-[4,3-a:3',4'-c]quinoxaline (12)

Compound **9b** (0.96 g, 0.003 mol) and diethyl oxalate (20 mL) were heated under reflux for 6 hours. The reaction mixture was cooled to room temperature and diluted with *n*-hexane (40 mL). The precipitated product was collected by filtration and washed with ether. Crystallization from the appropriate solvent afforded **12** as red needle crystals (Table I, II).

1-Ethoxycarbonylmethyl-6-(4-nitrophenyl)-[1,2,4]-ditriazolo[4,3-a:3',4'-c]quinoxaline (13)

Compound **9b** (0.96 g, 0.003 mol) and diethyl malonate (10 mL) were heated in an oil bath at 200°C for 2 hours. After cooling to room temperature, the reaction mixture was diluted with petroleum ether (b.p. 60-80°C, 30 mL) and stirred overnight. The precipitate formed was collected by filtration, washed with petroleum ether (b.p. 60-80°C) and crystallized from ethanol to afford **13** as reddish crystals (Table I, II).

***N,N',N'*-Triacetyl[4-hydrazino-1-(4-nitrophenyl)-[1,2,4]-triazolo[4,3-a]quinoxaline] (14)**

Acetic anhydride (10 mL) was added to **9b** (0.96 g, 0.003 mol) and the mixture was heated under reflux for 3 hours. The excess acetic anhydride was distilled off under diminished pressure. The semi-solid residue obtained was triturated with ice cold water (30 mL). The formed precipitate was filtered and crystallized from methylene chloride to furnish **15** as yellowish white crystals (Table I, II).

1-(4-Nitrophenyl)-4-[(2,5-dioxopyrrolidin-1-yl)amino]-[1,2,4]triazolo[4,3-a]quinoxaline (15)

A mixture of equimolar ratio of **9b** and succinic anhydride (0.003 mol of each) in glacial acetic acid (20 mL) was heated under reflux for 5 hours. After cooling, toluene (50 mL) was added with stirring. The formed precipitate was collected by filtration, washed with ether and crystallized from 1-butanol to yield **15** as red crystals (Table I, II).

1-(2-Carboxyphenyl)-6-(4-nitrophenyl)-[1,2,4]ditriazolo [4,3-a:3',4'-c]quinoxaline (16)

The title compound was prepared via the method described for **15** using compound **9b** and phthalic anhydride (0.003 mol of each). Crystallization from ethyl acetate afforded **16** as orange crystals (Table I, II).

4-(3,5-Dimethylpyrazol-1-yl)-1-(4-nitrophenyl)-[1,2,4]-triazolo[4,3-a]quinoxaline (17)

A solution of **9b** (0.96 g, 0.003 mol) and acetylacetone (0.45 mL, 0.0045 mol) in absolute ethanol (25 mL) was refluxed on a boiling water bath for 4 hours. After cooling to room temperature, the precipitated red cubic crystals were collected by filtration, washed with ether and recrystallized from the appropriate solvent (Table I, II).

RESULTS AND DISCUSSION

Chemistry

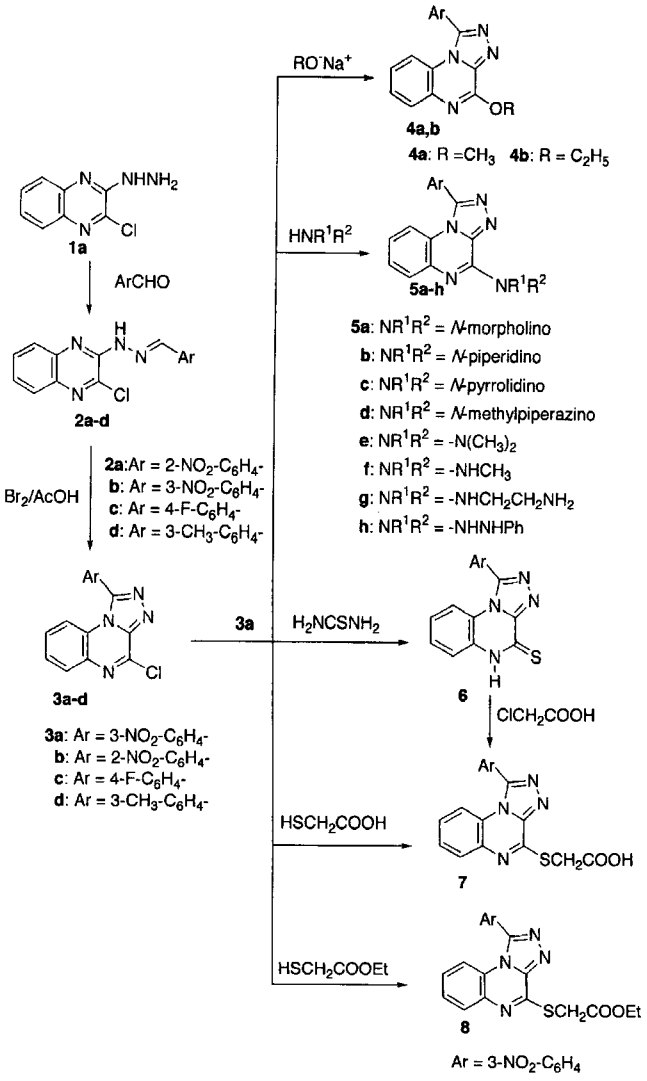
The intermediate **1a** and **1b** were prepared according to reported procedures (Cheesman, and Rafiq 1971; Krishnan *et al*., 2000; Sarges *et al*., 1990; Youssef *et al* 1976). Treatment of 2-chloro-3-hydrazinoquinoxaline (**1a**) with certain aromatic aldehydes gave 2-(arylidenehydrazino)-3-chloroquinoxalines (**2a-d**) which upon cyclization with bromine in acetic acid, afforded the corresponding 1-aryl-4-chloro-[1,2,4]triazolo[4,3-a]quinoxalines (**3a-d**). Reaction of **3a** with sodium methoxide and sodium ethoxide furnished the respective 4-alkoxy-1-(3-nitrophenyl)-[1,2,4]triazolo [4,3-a]quinoxalines (**4a, b**). Amination of **3a** with certain amines gave the respective 1-(3-nitrophenyl)-4-substituted-amino-[1,2,4]triazolo[4,3-a]quinoxalines (**5a-h**). Reaction of **3a** with thiourea afforded the isothiouronium intermediate (Campagne. and Mclaughin, 1983), which upon hydrolysis with alkali hydroxide yielded 1-(3-nitrophenyl)-[1,2,4]triazolo [4,3-a]quinoxalin-4(5*H*)-thione (**6**). Attempted alkylation of **6** with chloroacetic acid afforded the corresponding mercaptoacetic acid derivative **7**. The latter compound was also prepared by reacting **3a** and thioglycolic acid. Reaction of **3a** with ethyl thioglycolate afforded the ethyl thioglycolate derivative **8**.

On the other hand, treatment of 3-hydrazinoquinoxalin-2(1*H*-one (**1b**) with certain aromatic acids in phosphorus oxychloride followed by reaction with hydrazine hydrate afforded 1-aryl-4-hydrazino-[1,2,4]triazolo[4,3-a]quinoxalines (**9a,b**). In this one-pot reaction, the aromatic acid was first converted to its acid chloride, which subsequently reacted with the parent hydrazinoquinoxaline (**1b**) to give the corresponding acylhydrazino derivative in situ which underwent cyclodehydration followed by chlorination to yield the 4-chlorotriazoloquinoxaline. It finally reacts with excess hydrazine hydrate to afford the target compounds **9a,b**.

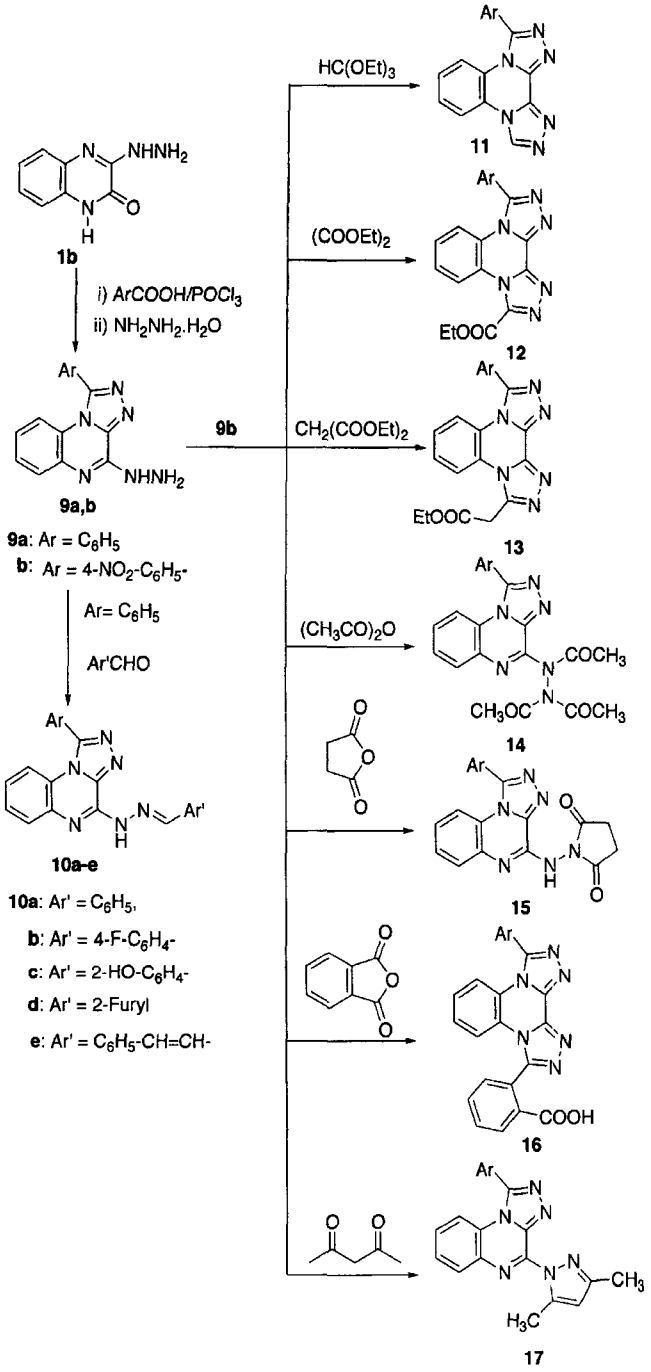
Compound **9a** was allowed to react with certain aromatic aldehydes to afford 4-(arylidenehydrazino)-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxalines (**10a-e**). Reaction of **9b** with certain esters or orthoesters furnished [1,2,4]ditriazolo[4,3-a:3',4-c]quinoxaline derivatives **11**, **12** and **13**.

Moreover, acetylation of **9b** with acetic anhydride afforded the triacetyl derivative **14** as confirmed by mass spectrum which showed successive loss of the three acetyl groups from the parent compound. The formation of the triacetyl derivatives **14** was unexpected since literature searching demonstrated that reaction of hydrazinoquinoxaline with acetic anhydride afforded the methyltriazolo derivative (Krishnan *et al*., 1994; Rashed *et al*., 1990; Campaigne and Mclaughlin, 1983). Reaction of **9b** with succinic anhydride afforded 1-(4-nitrophenyl)-4-[(2,5-dioxopyrrolidin-1-yl)amino]-[1,2,4 triazolo[4,3-a]quinoxaline (**15**). However, reaction of **9b** with phthalic anhydride furnished 1-(2-carboxyphenyl)-6-(6-(4-nitrophenyl)-[1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline (**16**) rather than 4-[(1,3-dioxo-isoindol-2-yl)amino]-1-(4-nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline. The formation of **16** was substantiated by chemical and spectral evidences, which proved the presence of free COOH. Thus, in ^{13}C-NMR, the peak at 166.2 ppm corresponds to the carbonyl group of the carboxylic acid function, while its IR showed a peak at 1740 cm^{-1} corresponding to C=O of the the acid. Finally, the mass spectrum demonstrated distinct fragmentation pattern due to loss of CO_2 molecule from the molecular ion peak. This previous way of cyclization was reported before in similar conditions The product of the latter reaction has been also obtained in similar reported situations (Badr *et al*., 1997). Finally, reaction of **9b** with acetylacetone afforded the respective pyrazolyl derivative **17**.

The synthetic pathway for the target compounds is depicted in the following schemes:



Scheme 1. Synthesis of compounds **1~8**



Scheme 2. Synthesis of compounds **9~17**.

Antimicrobial activity

Preliminary antibacterial and antifungal activity were performed for selected lead compounds against various types of bacteria and fungi, namely:

1. *Staphylococcus aureus* (Gram positive bacteria)
2. *Bacillus subtilis* (Gram positive spore-forming bacteria)
3. *Escherichia coli* (Gram negative bacteria)
4. *Pseudomonas aeruginosa* (very resistant Gram negative bacteria)
5. *Candida albicans* (a representative of fungi).

Table III. Results of antimicrobial activity, zones of inhibition (in mm)

Compound No.	*S. aureus*	*B. subtilis*	*E. coli*	*Ps. aeruginosa*	*C. albicans*
3a	34	22	8	–	–
4b	8	-	24	9	–
5a	–	–	–	–	–
5g	30	28	8	–	–
5h	28	16	9	–	–
6	–	–	–	–	22
9b	32	23	–	–	–
10e	–	–	–	–	–
11	12	7	–	–	–
13	–	–	–	–	8
14	–	–	–	–	–
15	22	6	–	–	–
17	-	-	19	14	–
T	30	28	22	8	–
N	–	–	–	–	26

T = Tetracycline standard disc.
N = Nystatin standard disc.
– = Inactive; inhibition zone < 7 mm

Materials

Culture media

Nutrient broth, Sabourauds broth and nutrient agar were the products of Oxoid ltd., England.

Methodology: the agar plate disc-diffusion technique (Collins, 1964)

Sterilized filter paper discs (6 mm in diameter) were wetted each with 10 μL of a solution of the tested compound containing 10 mg/mL in DMF and the discs were allowed to air dry. The discs were then placed onto the surface of agar plates (nutrient agar for bacteria and sabourauds dextrose agar for fungi) seeded with the test organism. Each plate contained 15 mL of the agar medium, previously seeded with 0.2 mL of 18 hours broth culture of each organism. The inoculated plates were incubated at 37°C for 48 hours and the inhibition zones were measured in mm. Discs impregnated with DMF were used as control. The antibacterial reference tetracycline and the antifungal reference nystatin discs were tested concurrently as a standard.

Results of antimicrobial activity

From Table III, it was found that in vitro antimicrobial testing revealed that compounds **5h** and **5g** possessed comparable antibacterial activity relative to the standard tetracycline, while **3a** and **9b** were even more potent than than standard. On the other hand, compound **6** possess-

ed a moderate antifungal activity against *C. albicans.*

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