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SYNTHESIS OF NEW QUINOXALINONE DERIVATIVES OF EXPECTED ANTIMICROBIAL ACTIVITY

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ABSTRACT

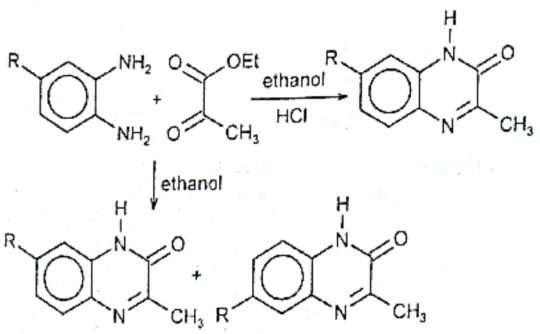
Using a series of chemical reactions, several quinoxalinone derivatives bearing chemically different moieties such as substituted acetanilido, arylideno, phthalimido, thiadiazolo and pyrazolo were prepared to study their antimicrobial activity. The structures of the new compounds were established using elemental and spectral analyses. The antimicrobial susceptibility testing of some novel compounds were carried out whereas some of them showed activity against Gram-positive rods only.

INTRODUCTION

Several biological activities are linked to quinoxalinone derivatives such as central serotoninmimetic[1], antitumor[2,3], antiasthmatic[4], antiviral[5], anti-inflammatory[6] and antiulcer[7]. In addition, other quinoxalinone derivatives have been found to possess antimicrobial activity[8-11]. In the present work, new quinoxalinone derivatives which bearing various chemical moieties were synthesized with the aim to examine their antimicrobial activity. Furthermore, the difference in the structure of these moieties facilitates the investigation of their effects as antimicrobial agents.

DISCUSSION

In this work, the designed quinoxalinone derivatives were prepared as outlined in schemes I and II. The starting compound[12] 7-chloro-3-methyl-2(1H)-quinoxalinone (**1**) was obtained via condensation of 4-chloro-1,2-diaminobenzene with ethyl pyruvate in ethanol containing 1 mol equivalent of HCl at room temperature. It is important to note that when the 4-monosubstituted-1,2-diaminobenzene was condensed with pyruvic acid or its ester, a mixture of 6/7 substituted isomers[13-15] was obtained under neutral condition while the acidic condition[16,17] lead to the formation of 7-isomer as a major product as shown below:



Thus, the acidic condition is preferable due to HCl can protonate the more basic 2-amino group of 4-chloro-1,2-diaminobenzene preventing its condensation with the carbonyl function of ethyl pyruvate which favors the formation of the 7-isomeric product.

The key starting compound **1** was easily alkylated with the active halogen compounds, namely, chloro-acetanilides[18,19] and ethyl bromoacetate through heating the reactants in dry acetone containing anhydrous K_2CO_3 to afford the novel 7-chloro-3-methyl-1-(substitutedphenylamino-carbonylmethyl)-2(1H)-quinoxalinones (**2a-e**) and the ester derivative **3** in high yields, respectively (Scheme I).

The formation of the new acid hydrazide **4** was achieved via heating the ester **3** and hydrazine hydrate in ethanol for 3 h.

Condensation of the hydrazide **4** with different aromatic aldehydes was conducted by heating the reactants in ethanol containing catalytic amount of glacial acetic acid to afford the novel hydrazone derivatives **5a-d** (Scheme I).

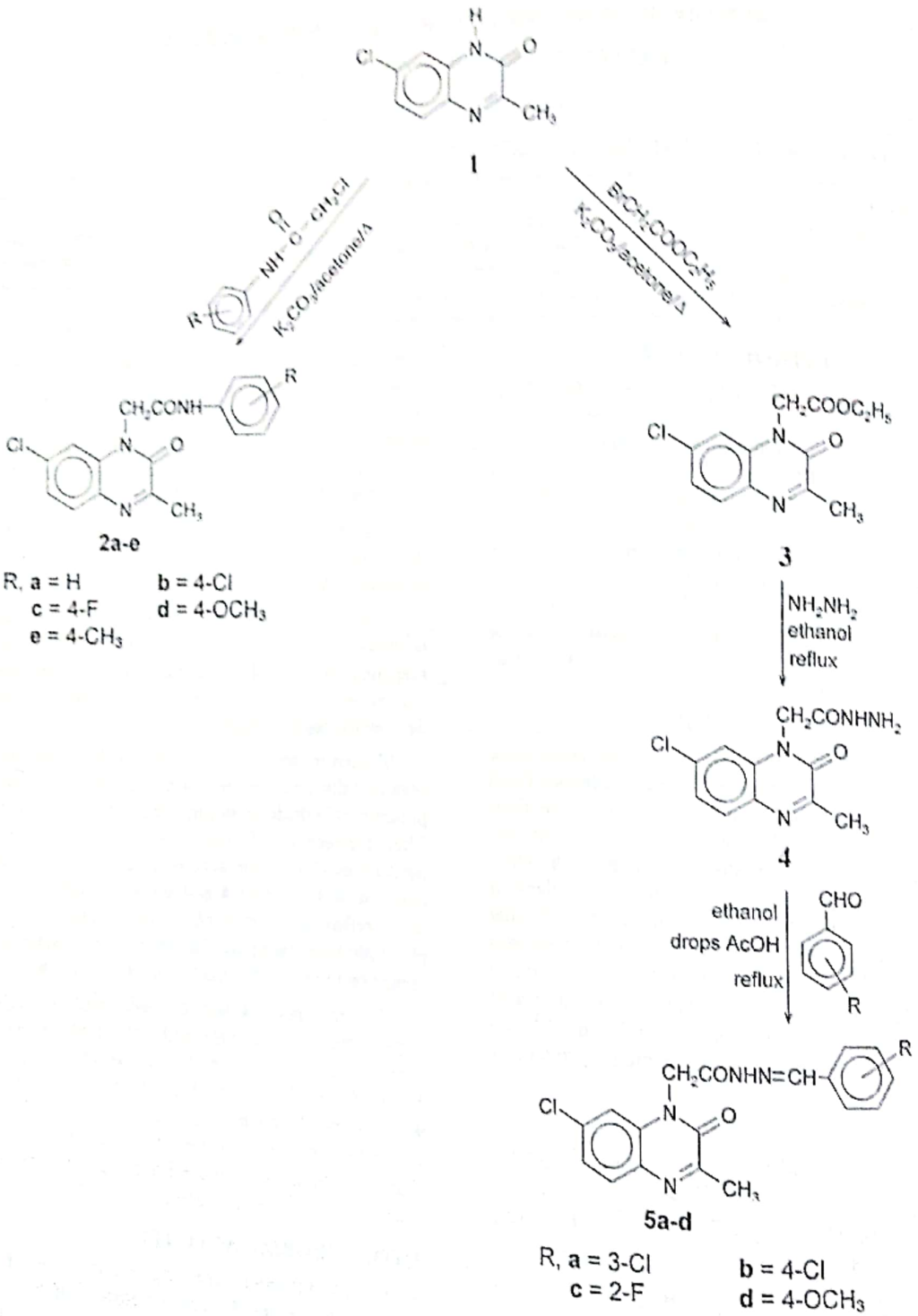
Moreover, the new phthalimido derivative **6** was obtained through condensation of the hydrazide **4** with phthalic anhydride in boiling acetic acid (Scheme II). The formation of the novel thiosemicarbazide derivatives (**7a,b**) was accomplished through addition reaction of hydrazide **4** and different isothiocyanates in refluxing ethanol. In addition, the phenylthiosemicarbazide **7a** was cyclized under acidic condition to afford the thiadiazole derivative **8**.

The hydrazide **4** was cyclized with β-diketones namely ethyl acetoacetate and acetylacetone via 2+3 cycloaddition reaction in refluxing ethanol containing drops of glacial acetic acid to afford the pyrazolinone **9** and pyrazole **10** in high yields, respectively. The structures of the novel quinoxalinones were characterized using elemental analyses, IR, ^{1}H NMR and mass spectroscopic methods.

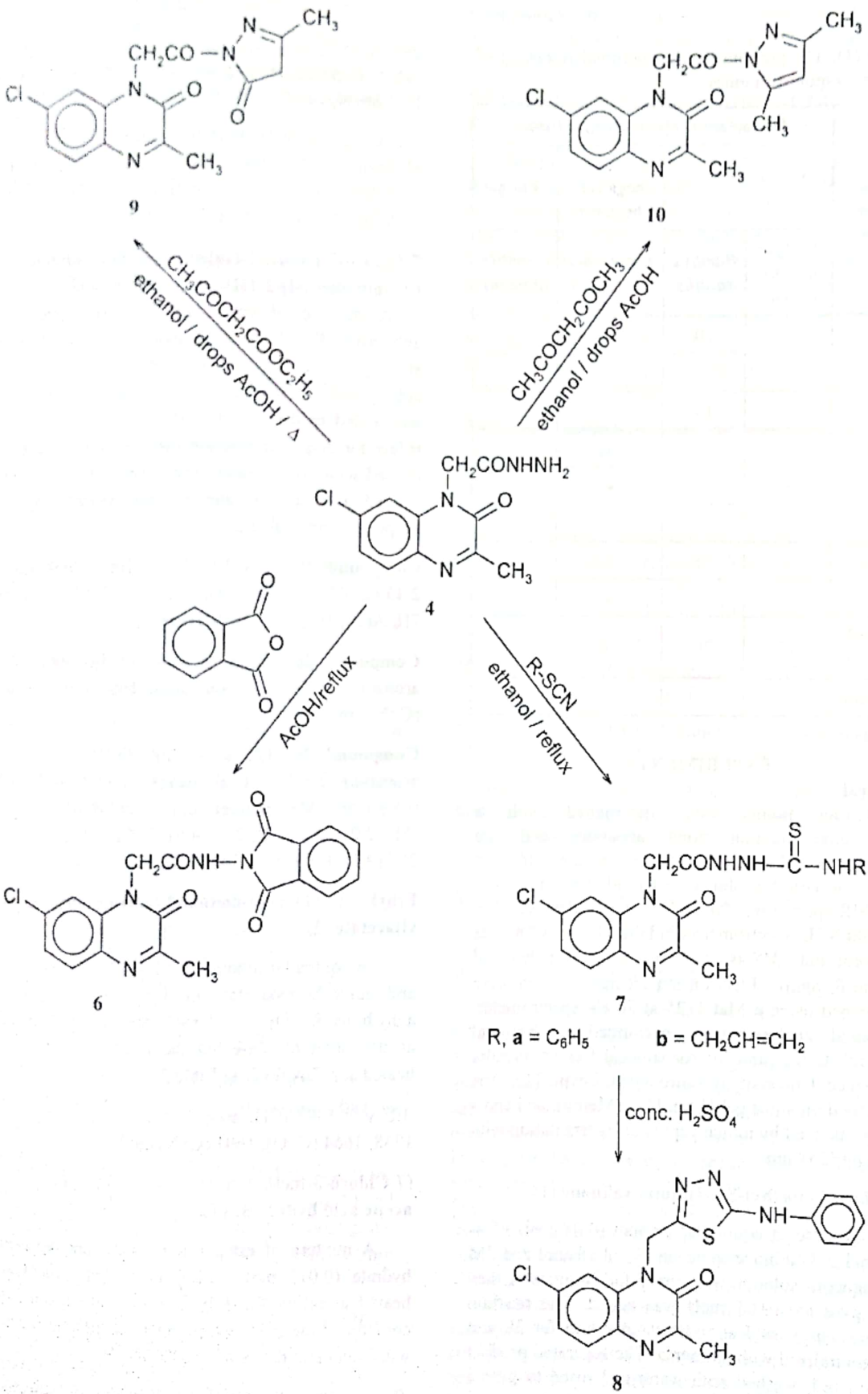
ANTIMICROBIAL ACTIVITY

The antimicrobial screening of compounds **1**, **2c**, **3**, **4**, **5b**, **6**, **7a** and **8** against Gram-positive, Gram-negative bacteria and fungi was carried out using the disk agar diffusion method[20].

It was noticed that compounds **1** and **3** have moderate antibacterial activity while compounds **4** and **7a** have nearly the same activity as that of ciprofloxacin against Gram-positive rods only (Table 1). Compounds **2c**, **5b**, **6** and **8** have no antibacterial activity at all against both Gram- positive and Gram-negative bacteria. All the tested compounds have no antifungal activity.



Scheme 1



Scheme II

Table (1): The preliminary antimicrobial screening of some new quinoxalinones

Test compounds	Diameter of inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*
1	-	10	-	-
2c	-	-	-	-
3	-	15	-	-
4	-	19	-	-
5b	-	-	-	-
6	-	-	-	-
7a	-	18	-	-
8	-	-	-	-
Ciprofloxacin	17	20	14	-
Nystatin	-	-	-	14

EXPERIMENTAL

General

Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Bruker or Testscan Shimadzu FT 8000 spectrometer. ^1H NMR spectra were recorded on a Bruker AC 250 and 500 MHz spectrometers in DMSO-d_6 or $CDCl_3$ as a solvent and *TMS* as an internal standard (Chemical shift in δ, ppm). Electron impact mass spectra were determined using a Mat 1125 at 70 eV spectrometer. Elemental analyses were performed at National Research Center and Microanalytical Center, Faculty of Science, University of Cairo, Giza, Egypt. TLC was performed on silica gel G for TLC (Merck), and spots were visualized by iodine vapors or by irradiation with UV light (254 nm).

7- chloro-3- methyl-2(1H)-quinoxalinone (1).

A mixture of equimolar amount (0.01 mol) of 4-chloro-1,2-diaminobenzene in 20 ml ethanol and 2M HCl aqueous solution was stirred for 20 minutes, then ethyl pyruvate (0.01 mol) was added. The reaction mixture was stirred at room temperature for 3h and then neutralized with ammonia. The separated product was filtered, washed with water and dried to give a crude product (60% yield) which by repeated fractional recrystallization from benzene gave pure product, m.p.°C 268-270, 40% yield, (reported m.p.°C 265-267)[19]. Analysis for $C_9H_7ClN_2O$ (194.59), % calcd. C, 55.54, H, 3.62, N, 14.39; % found C, 55.50, H, 3.60, N, 14.57.

IR: ν = 3311 (NH), 3151 (CH, aromatic), 2986 (CH, aliphatic), 1673 (C=O), 1607 (C=N) cm^{-1}. ^1HNMR (250 MHz, DMSO-d6) δ: 2.42 (s, 3H, CH_3), 7.28 – 7.74 (m, 3H, ArH), 12.34 (s broad, 1H, NH) ppm.

7-Chloro-3-methyl-1-(substitutedphenylamino-carbonylmethyl)-2(1H)-quinoxalinones (2a-e).

A mixture of compound **1** (0.003 mol) and anhydrous K_2CO_3 (0.0032 mol) was heated while stirring in 20 ml dry acetone for 15 min. The appropriate chloroacetanilide derivative (0.003 mol) was added to the stirred mixture and then heated at reflux for 3 h. The reaction mixture was cooled and diluted with 20 ml water. The separated product was filtered, washed with water and recrystallized from the proper solvent (Table 2).

Compound 2b: ^1HNMR (250 MHz, DMSO-d6) δ: 2.45 (s, 3H, CH_3), 5.12 (s, 2H, CH_2), 7.37 – 7.82 (m, 7H, ArH), 10.61 (s, 1H, NH) ppm.

Compound 2c: IR: ν = 3257 (NH), 3066 (CH, aromatic), 2959 (CH, aliphatic), 1665 (C=O), 1604 (C=N) cm^{-1}.

Compound 2d: IR: ν = 3260 (NH), 3071 (CH, aromatic), 2960 (CH, aliphatic), 1660 (C=O), 1601 (C=N) cm^{-1}. MS: m/z (rel. int.) = 359 (M^++1, 3.5), 358 (M^+, 2.0), 357 (7.4), 237 (4.6), 236 (3.1), 235 (13.7), 207 (9.2), 179 (28.0), 144 (3.8), 123 (100.0).

Ethyl (7-chloro-3-methyl-2-(1H)-quinoxalinon-1-yl)acetate (3).

An equimolar amount (0.01 mol) of compound **1** and ethyl bromoacetate in dry acetone containing anhydrous K_2CO_3 (0.012 mol) was reacted similarly as described for **2a-e** but the reaction mixture was heated at reflux for 2 h (Table 2).

IR: ν = 3083 (CH, aromatic), 2957 (CH, aliphatic), 1738, 1664 (C=O), 1601 (C=N) cm^{-1}.

(7-Chloro-3-methyl-2(1H)-quinoxalinon-1-yl) acetic acid hydrazide (4).

A mixture of ester **3** (0.01 mol) and hydrazine hydrate (0.012 mol, 100%) in 40 ml ethanol was heated at reflux for 3 h. The reaction mixture was cooled and the separated product was filtered, washed with water and recrystallized (Table 2).

IR: ν = 3303, 3250 (NH, NH_2), 3051 (CH, aromatic), 2924 (CH, aliphatic), 1654 (C=O), 1600 (C=N) cm^{-1}. ^1HNMR (250 MHz, DMSO-d6) δ: 2.42 (s, 3H, CH_3),

δ 5.31 (s, 2H, NH_2, exch.), 4.86 (s, 2H, CH_2), 7.27 – 7.77 (m, 3H, ArH), 9.36 (s, 1H, NH, exch.)

7-Chloro-3-methyl-1-(substitutedbenzylidene-hydrazinocarbonylmethyl)-2(1H)-quinoxalinones (5a-d).

Equimolar amounts (0.002 mol) of the acid hydrazide **4** and the appropriate aromatic aldehydes were dissolved in 30 ml ethanol containing 2 ml glacial acetic acid and the mixture was heated at reflux for 2 h. The separated product after cooling was filtered, washed with ethanol and recrystallized from the proper solvent (Table 2).

Compound 5a: IR: ν = 3187 (NH), 3064 (CH, aromatic), 2950 (CH, aliphatic), 1691, 1659 (C=O), 1599 (C=N) cm^{-1}.

Compound 5b: ^{1}HNMR (250 MHz, DMSO-d6) δ: 2.46 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 7.51 – 7.81 (m, 7H, ArH), 8.07 (s, 1H, CH=N), 11.91 (s broad, 1H, NH) ppm.

Compound 5d: MS: m/z (rel. int.) = 386 (M^++1, 4.6), 384 (12.2), 238 (5.2), 237 (27.4), 236 (12.5), 235 (100.0), 207 (30.6), 181 (17.5), 179 (60.7), 150 (68.5).

7-Chloro-3-methyl-1-(phthalimidoaminocarbonylmethyl)-2(1H)-quinoxalinone (6).

A mixture of the acid hydrazide **4** (0.002 mol) and phthalic anhydride (0.002 mol) in 20 ml glacial acetic acid was heated at reflux for 3 h. the reaction mixture was cooled and the separated product was filtered and recrystallized (Table 3).

IR: ν = 3221 (NH), 3027 (CH, aromatic), 2923 (CH, aliphatic), 1795, 1736, 1640 (C=O), 1598 (C=N) cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d6) δ: 2.47 (s, 3H, CH_3), 5.22, 5.23 (two s, 2H, CH_2), 7.49 – 7.98 (m, 7H, ArH), 11.2 (s, 1H, NH) ppm. MS: m/z (rel. int.) = 398 (M^++1, 3.2), 397 (M^+, 12.1), 381 (2.6), 380 (5.6), 238 (11.6), 237 (24.8), 236 (18.4), 235 (100.0).

N^1-[(7-chloro-3-methyl-2(1H)-quinoxalinon-1-yl)-methylcarbonyl]-N^4-substituted thiosemicarbazide (7a,b).

A mixture of the hydrazide **4** (0.004 mol) and the appropriate isothiocyanate (0.004 mol) in 40 ml ethanol was heated at reflux for 2 h. After cooling, the separated product was filtered and recrystallized from the proper solvent (Table 3).

Compound 7a: IR: ν = 3253 (NH), 2962 (CH, aliphatic), 1649 (C=O), 1600 (C=N) cm^{-1}. ^{1}HNMR (250 MHz, DMSO-d6) δ: 2.52 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 7.34 – 7.78 (m, 8H, ArH), 9.77, 9.80 (s broad, 2H, 2NH), 10.51 (s, 1H, NH) ppm.

Compound 7b: IR: ν = 3212 (NH), 3057 (CH, aromatic), 2919 (CH, aliphatic), 1692, 1676 (C=O), 1603 (C=N) cm^{-1}.

7-Chloro-3-methyl-1-(5-phenylamino-1,3,4-thiadiazol-2-yl)-2(1H)-quinoxalinone (8).

The phenylthiosemicarbazide **7a** (0.002 mol) was dissolved in 10 ml concentrated H_2SO_4 while cooling and then allowed to stand for 1 h. The reaction mixture was poured on crushed ice with continuous stirring and then neutralized with NH_4OH. The separated product was filtered, washed with water and recrystallized (Table 3).

MS: m/z (rel. int.) = 386 (M^++2, 11.0), 385 (M^++1, 39.6), 384 (M^+, 24.1), 383 (100.0), 190 (69.9), 179 (20.3), 136 (24.1), 77 (49.4).

7-Chloro-3-methyl-1-(3-methyl-5-oxo-1H-pyrazolin-1-yl carbonylmethyl)-2(1H)-quinoxalinone (9).

To a solution of the hydrazide **4** (0.002 mol) in 30 ml ethanol containing 2 ml glacial acetic acid, an equimolar amount of ethyl acetoacetate was added. The reaction mixture was heated at reflux for 5 h, then concentrated, cooled and added to 20 ml ice-cold water. The separated product was filtered, washed with water and recrystallized (Table 3).

IR: ν = 3083 (CH, aromatic), 2985 (CH, aliphatic), 1736, 1698, 1662 (C=O), 1602(C=N) cm^{-1}.

7-Chloro-3-methyl-1-(3,5-dimethyl-1H-pyrazol-1-ylcarbonylmethyl)-2(1H)-quinoxalinone (10).

An equimolar amount (0.002 mol) of the acid hydrazide **4** and acetylacetone was reacted similarly as described for preparation of **9** (Table 3).

^{1}HNMR (500 MHz, DMSO-d6) δ: 2.28 (s, 3H, CH_3), 3.34 (s, 6H, $2CH_3$), 5.77, 5.78 (two s, 2H, CH_2), 6.32 (s, 1H, CH of pyrazole), 7.79-7.91 (m, 3H, ArH) ppm.

ANTIMICROBIAL ACTIVITY

The chosen new quinoxalinones **1, 2c, 3, 4, 5b, 6,7a** and **8** were dissolved in DMF at concentration 10mg/ml. Ciprofloxacin (disc, 5μg, Oxoid, England) and nystatin were used as standards. The discs were saturated with 5 μg from the newly synthesized quinoxalinones, and dried in oven at 60°C before loading on the surface of the seeded Mueller Hinton agar medium[26]. The agar was seeded with *Staphylococcus aureus* as Gram-positive cocci and *Bacillus subtilus* as Gram-positive rods, *Esherichia coli* as Gram-negative rods as well as *Candida albicans* as fungi. The plates were incubated at 37°C for 24h and the diameters of the inhibition zone were measured in mm (Table 1). Bacterial and fungal strains were isolated and identified by Department of Microbiology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Table 2. Physical data for compounds 2a-e, 3, 4 and -5a-d.

Comp. NO.	R	Recryst-allization solvent	Yield %	m.p. °C	Mol. Form. (M. wt.)	Analyses% (Caled./Found)		
						C	H	N
2a	H	ethanol	93	227-29	$C_{17}H_{14}ClN_3O_2$ (327.74)	62.29 62.02	4.30 4.31	12.82 12.85
2b	4-Cl	dioxane/ H_2O	95	261-63	$C_{17}H_{13}Cl_2N_3O_2$ (362.18)	56.37 56.25	3.61 3.65	11.60 11.38
2c	4-F	ethanol	90	264-66	$C_{17}H_{13}ClFN_3O_3$ (345.72)	59.05 59.07	3.78 3.80	12.15 12.26
2d	4-OCH_3	dioxane/ H_2O	94	248-50	$C_{18}H_{16}ClN_3O_3$ (357.76)	60.42 60.24	4.50 4.88	11.74 11.94
2e	4-CH_3	ethanol	90	295-97	$C_{18}H_{16}ClN_3O_2$ (341.76)	63.25 63.10	4.71 4.72	12.29 12.44
3	-	ethanol/ H_2O	88	118-20	$C_{13}H_{13}ClN_2O_3$ (280.68)	55.62 55.56	4.66 4.57	9.97 9.67
4	-	ethanol	80	229-30	$C_{11}H_{11}ClN_4O_2$ (266.65)	49.54 49.60	4.15 4.32	21.00 21.10
5a	3-Cl	dioxane/ H_2O	85	291-93	$C_{18}H_{14}Cl_2N_4O_2$ (389.20)	55.54 55.30	3.62 3.58	14.39 14.52
5b	4-Cl	dioxane/ H_2O	95	304-06	$C_{18}H_{14}Cl_2N_4O_2$ (389.20)	55.54 55.93	3.62 4.10	14.39 14.38
5c	2-F	dioxane/ H_2O	90	268-70	$C_{18}H_{14}ClFN_4O_2$ (372.78)	57.99 58.00	3.78 3.72	15.03 14.85
5d	4-OCH_3	dioxane/ H_2O	92	284-85	$C_{19}H_{17}ClN_4O_3$ (384.78)	59.30 59.46	4.45 4.41	14.55 14.21

Table 3. Physical data for compounds 6, 7a,b and 8-10.

Comp. NO.	R	Recryst-allization solvent	Yield %	m.p. °C	Mol. Form. (M. wt.)	Analyses% (caled./found)		
						C	H	N
6	-	acetic acid	82	297-99	$C_{19}H_{13}ClN_4O_4$ (396.75)	57.55 57.77	3.30 3.83	14.11 14.38
7a	C_6H_5	dioxane/ H_2O	89	258-60	$C_{18}H_{16}ClN_5O_2S$ (401.83)	53.79 53.46	4.01 4.21	17.42 17.55
7b	CH_2-$CH{=}CH_2$	ethanol	82	240-42	$C_{15}H_{16}ClN_5O_2S$ (365.80)	49.24 48.94	4.40 4.73	19.14 19.18
8	-	dioxane/ H_2O	70	278-79	$C_{18}H_{14}ClN_5O_2S$ (383.82)	56.32 56.20	3.67 3.81	18.24 18.06
9	-	ethanol/ H_2O	68	135-37	$C_{15}H_{13}ClN_4O_3$ (332.71)	54.14 53.90	3.93 4.32	16.83 16.65
10	-	ethanol/ H_2O	70	177-79	$C_{16}H_{15}ClN_4O_2$ (330.73)	58.10 58.12	4.56 4.27	16.93 16.85

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تشييد بعض مشتقات الكينوكسالينون الجديدة والمتوقع أن يكون لها فاعلية ضد الميكروبات

أسامة إبراهيم الصباغ

قسم الكيمياء الطبية – كلية الصيدلة – جامعة الزقازيق – الزقازيق – مصر.

تم تحضير العديد من مشتقات الكينوكسالينون الجديدة والمحملة بأجزاء مختلفة كيميائيا مثل مستبدل الأسيتانيليدو والأريليدينو وفثاليميدو والثياديازولو والبيرازولو لدراسة تأثير هذه الأجزاء على الفاعلية كمضادات للميكروبات. ولقد تم إثبات التركيبات البنائية للكينوكسالينونات الجديدة باستخدام التحاليل الدقيقة للعناصر وكذلك الطيفية.

ولقد تم إجراء اختبارات على بعض المركبات الجديدة كمضادات للميكروبات حيث أظهر بعضها فاعلية ضد الميكروبات العصيية الموجبة الجرام فقط.