DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL [1,2,4]TRIAZOLO[4,3-A] QUINOXALINONES

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ABSTRACT

A series of novel [1,2,4]triazolo[4,3-*a*]quinoxalinone derivatives have been synthesized. The structures of the newly synthesized compounds were confirmed by elemental analysis and different spectral techniques. The *in vitro* antimicrobial screening of some selected compounds was carried out using the diffusion agar technique. Compound **14_c** was found to be the most active antibacterial agent against *Escherichia coli using* gentamicin as reference drug. All the tested compounds showed no activity against *Candida albicans* and *Pseudomonas aerugeosa*.

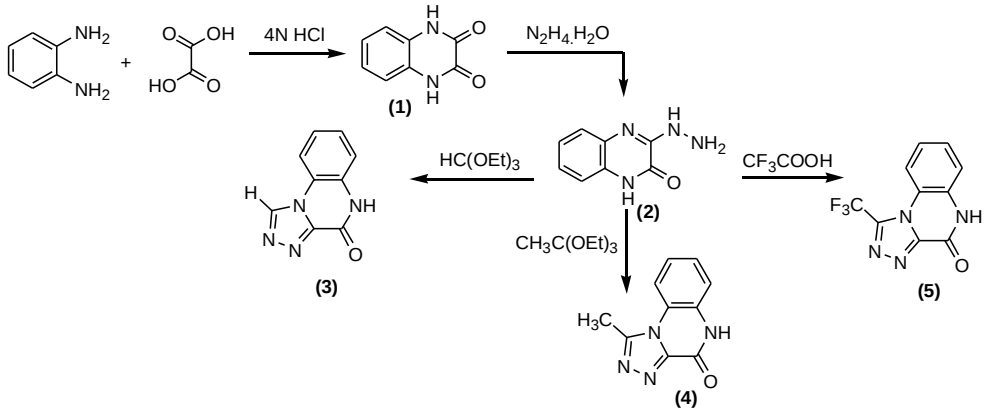
Introduction

Although antibacterial agents are life saving drugs, yet they suffer from serious drawbacks. The most important of which is anaphylaxis and developing resistance which implement a potential global health crisis. It is recommended to use new antibacterial agents with enhanced broad-spectrum potency. Therefore, recent efforts have been directed toward exploring novel antibacterial agents. Quinoxaline derivatives are important components of several pharmacologically active compounds [Tandon *et al*., 2006; Sarges *et al*., 1990; Sakata *et al*., 1988; Arthur *et al*., 2005; Seitz *et al*., 2002; Szekelyhidi *et al*., 2005] . Although rarely described in nature, synthetic quinoxaline ring is part of number of antibiotics such as echinomycin and actinomycin, which are known to inhibit the growth of Gram-positive bacteria and are also active against various transplantable tumors [Myers *et al*., 2003; Iwashita *et al.,* 2005*;* Morokata *et al.,* 2004]. Substituted quinoxaline have received considerable attention during last decade as they are endowed with variety of biological activities and have wide range of therapeutic properties such as antibacterial, antiviral, anticancer, antifungal, anthelmintic, and insecticidal [Badran *et al*., 2003; Catarzi *et al.,* 2004*;* Gali-Muhtasib *et al.,* 2005*;* Perez-Melero *et al.,* 2004*;* Kim *et al.,* 2004*;* Toshima *et al.,* 2004]. Relying on this evidence we became motivated to synthesize some novel [1,2,4]triazolo[4,3-*a*]quinoxalinone derivatives in an attempt to find an effective antimicrobial agent.

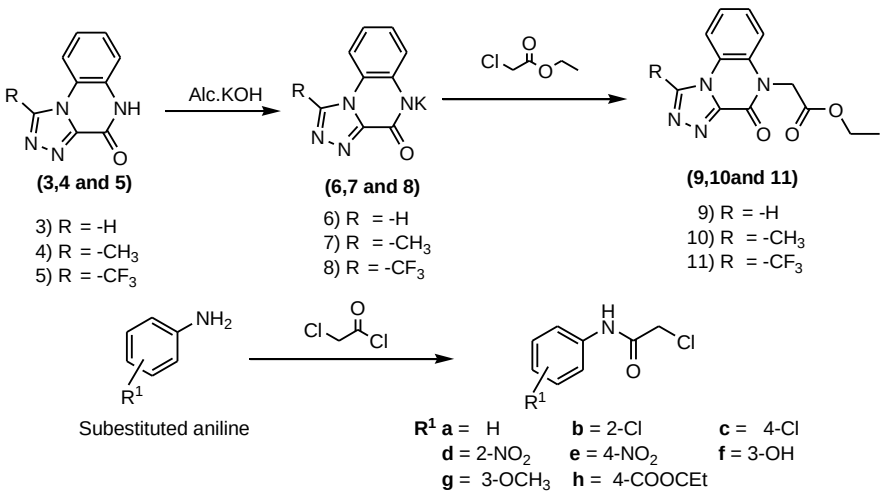
Results and Discussion

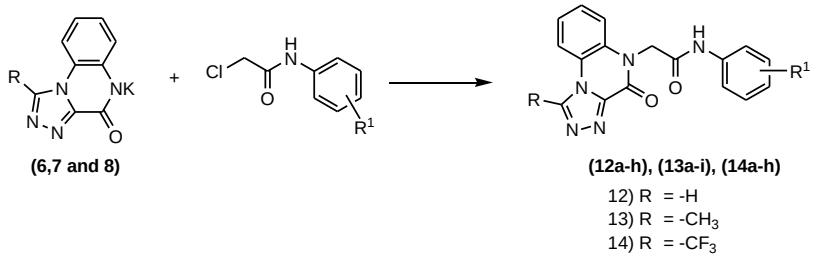
The present work involves preparation of some novel [1,2,4]triazolo[4,3-*a*]quinoxalinone derivatives. Achieving this goal was made by adopting the synthetic pathway depicted in **schemes I and II**. The main intermediate in this work is 3-hydrazinylquinoxalinone **2** which has been synthesized in two steps starting from O-phenylenediamine by treating it with oxalic acid to produce quinoxalinedione **1**. Reacting **1** with hydrazine hydrate afforded the hydrazinylquinoxalinone **2**. Triazoloquionxalinone derivatives **3, 4** and **5** have been prepared from compound **2** using established procedures **Scheme I**. Potassium salts **6, 7** and **8** were obtained after treatment of compounds **3, 4** and **5** with alcoholic KOH in quantitative yields. Reaction of potassium salts **6, 7** and **8** with ethylchloroacetate gave the corresponding esters **9, 10** and **11** respectively**.** Reaction of potassium salts **6, 7** and **8** with chloroacetanilides produced the corresponding anilides **12**a-h, **13**a-i and **14**a-h **Scheme II.** The IR spectra of these compounds are characterized by appearance of amid carbonyl at 1681-1681cm^{-1} and amidic -NH at 3245- 3448 cm^{-1}.

The structural assignments to the new compounds were based on their elemental analysis and spectral (IR, ^{1}HNMR and mass) data.



Scheme I





Scheme II

Experimental

All melting points were taken on Stuart SMP3 digital melting point apparatus and Gallen Kamp apparatus and were uncorrected.IR spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer at Microanalytical Center, Cairo University. The ^{1}H NMR spectra were recorded in DMSO-d_6 at 300 MHz on a Varian Mercury VXR-300 NMR spectrometer at Research Services Unit, Faculty of Science, Cairo University. Chemical shifts were related to those of the solvent. TMS was used as a standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at Regional Center for Mycology and Biotechnology, Al-Azhar University. Microanalysis was carried out at Microanalytical Center, Cairo University. Reactions progresses were monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp.

According to certain reported procedures, the following derivatives were prepared:

Quinoxaline-2,3(*1H,4H*)-dione(Romer, 2009), 3-hydrazinylquinoxalin-2(1*H*)-one(Newbold *et al*., 1948), [1,2,4]triazolo[4,3-*a*]quinoxalin-4(5*H*)-one(Galal *et al*., 2013), 1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4(5*H*)-one(Rashed *et al*., 1990) and 1-(trifluoromethyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4(5*H*)-one (Bharat *et al*., 1988)**,** Chloroacetailides (El-Said *et al*., 1991).

Potassium salt of [1,2,4]triazolo[4,3-a]quinoxalin-4(5H)-one (6),
Potassium salt of 1-methyl-[1,2,4]triazolo[4,3-a]quinoxalin-4(5H)-one (7)
and Potassium salt of 1-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4(5H)-one (8):

To a solution of the appropriate (1,2,4)-triazolo[4,3-a]quinazolinone of type **3**, **4** and **5** (0.01mol each) in absolute ethanol, solution KOH (0.01mol) in absolute ethanol was added and the reaction mixture was heated to 78°C with stirring for 15minutes. The produced solid was collected by filtration, washed several times with absolute ethanol and dried producing the target compounds in quantitative yield.

Ethyl 2-(4-oxo-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)acetate (9):
Ethyl 2-(1-methyl-4-oxo-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)acetate (10):
Ethyl2-(4-oxo-1-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)acetate(11):

General procedure

In a conical flask equimolar quantities (0.01mol each) of the suitable salt **6** or **7** or **8** and ethyl chloroacetate were suspended in (20ml) DMF and heated on water bath for 3h. The reaction mixture was poured onto ice water (200 ml), and stirred for 30min. The obtained solid was filtered and crystallized from acetone: methanol (1:5) mixture.

2-(4-oxo-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)-N-substituted phenylacetamide(12a-h):

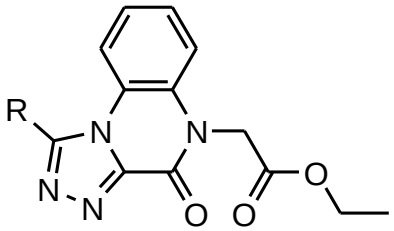
2-(1-methyl-4-oxo-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)-N-substituted phenylacetamide (13a-i):

2-(4-oxo-1-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]quinoxalin-5(4H)-yl)-N-substituted phenylacetamide (14a-h):

General procedure

Equimolar quantities of the appropriate potassium salt **6 or 7** or **8** (0.01 mol) and the appropriate chloroacetatnilide (0.01 mol) in DMF (20 ml) were heated on water bath for 3h. The reaction mixture was poured onto ice water (200 ml), and stirred for 30 min. The obtained solid was filtered and crystallized from aqueous ethanol.

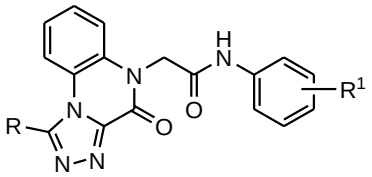
Table 1: Physical and analytical data of the synthesized compounds:



(9,10and 11)

No	*R*	*Yield %*	*m.p. °C*	*M. Formula/ M. Wt*	*Analysis % Calcd /found*		
					C	*H*	*N*
9	H	35	210-12	$C_{13}H_{12}N_4O_3$ 272.2	57.35 57.35	4.44 4.34	20.58 20.60
10	CH_3	76	261-62	$C_{14}H_{14}N_4O_3$ 286.2	58.73 58.57	4.93 5.21	19.57 19.39
11	CF_3	83	220-22	$C_{14}H_{11}F_3N_4O_3$ 340.2	49.42 49.22	3.26 3.15	16.47 16.50

Table 2: Physical and analytical data of the synthesized compounds:



(12a-h), (13a-i), (14a-h)

No	*R*	*R^1*	*m.p. °C*	*Yield %*	*M. Formula/M.Wt*	*Analysis % Calcd/found*		
						C	*H*	*N*
12a	H	H	316-317	70	$C_{17}H_{13}N_5O_2$	63.94	4.10	21.93
					319.3	64.00	4.31	21.54
12b	H	2-Cl	325-326	65	$C_{17}H_{12}ClN_5O_2$	57.72	3.42	19.80
					353.7	57.83	3.46	19.96
12c	H	4-Cl	314-315	82	$C_{17}H_{12}ClN_5O_2$	57.72	3.42	19.80
					353.7	57.85	3.48	19.93
12d	H	2-NO_2	284-386	63	$C_{17}H_{12}N_6O_4$	56.05	3.32	23.07
					364.3	56.21	3.10	22.98
12e	H	4-NO_2	345-346	57	$C_{17}H_{12}N_6O_4$	56.05	3.32	23.07
					364.3	56.19	3.39	23.30
12f	H	3-OH	264-266	64	$C_{17}H_{13}N_5O_3$	60.89	3.91	20.89
					335.3	61.07	3.94	21.04
12g	H	3-OCH_3	292-295	63	$C_{18}H_{15}N_5O_3$	61.89	4.33	20.05
					349.3	61.97	4.39	20.13
12h	H	4-COOEt	303-304	85	$C_{20}H_{17}N_5O_4$	61.38	4.38	17.89
					391.3	61.47	4.46	18.02
13a	CH_3	H	285-287	51	$C_{18}H_{15}N_5O_2$	64.86	4.54	21.01
					333.3	64.97	4.59	21.13
13b	CH_3	2-Cl	332-333	66	$C_{18}H_{14}ClN_5O_2$	58.78	3.84	19.04
					367.7	58.63	3.89	19.17
13c	CH_3	4-Cl	345-346	56	$C_{18}H_{14}ClN_5O_2$	58.78	3.84	19.04
					367.7	58.86	3.86	19.17
13d	CH_3	2-NO_2	298-300	65	$C_{18}H_{14}N_6O_4$	57.14	3.73	22.21
					378.3	57.25	3.81	22.36
13e	CH_3	4-NO_2	330-331	63	$C_{18}H_{14}N_6O_4$	57.14	3.73	22.21
					378.3	57.05	3.65	22.09
13f	CH_3	3-OH	327-328	55	$C_{18}H_{15}N_5O_3$	61.89	4.33	20.05
					349.3	61.98	4.35	20.16
13g	CH_3	3-OCH_3	270-272	53	$C_{19}H_{17}N_5O_3$	62.80	4.72	19.27
					363.3	62.87	4.78	19.35
13h	CH_3	4-COOEt	300-302	70	$C_{21}H_{19}N_5O_4$	62.22	4.72	17.27
					405.4	62.19	4.53	17.32
13i	CH_3	4-COOMe	291-293	69	$C_{20}H_{17}N_5O_4$	61.38	4.38	17.89
					391.3	61.09	4.42	17.63
14a	CF_3	H	283-284	58	$C_{18}H_{12}F_3N_5O_2$	55.82	3.12	18.08
					387.3	55.91	3.18	18.11
14b	CF_3	2-Cl	237-238	56	$C_{18}H_{11}ClF_3N_5O_2$	51.26	2.63	16.61
					421.7	51.38	2.97	16.34
14c	CF_3	4-Cl	283-285	55	$C_{18}H_{11}ClF_3N_5O_2$	51.26	2.63	16.61
					421.7	51.42	2.60	16.69
14d	CF_3	2-NO_2	279-280	60	$C_{18}H_{11}F_3N_6O_4$	50.01	2.56	19.44
					432.3	50.21	2.45	19.31
14e	CF_3	4-NO_2	296-297	65	$C_{18}H_{11}F_3N_6O_4$	50.01	2.56	19.44
					432.3	50.13	2.62	19.57

14f	CF_3	3-OH	288-282	71	$C_{18}H_{12}F_3N_5O_3$ 403.3	53.60 53.71	3.00 2.98	17.36 17.43
14g	CF_3	3-OCH_3	270-271	66	$C_{19}H_{14}F_3N_5O_3$ 417.3	54.68 54.81	3.38 3.35	16.78 16.89
14h	CF_3	4-COOEt	245-246	60	$C_{21}H_{16}F_3N_5O_4$ 459.3	54.91 54.99	3.51 3.59	15.25 15.34

Table 3: Spectral data of the prepared compounds:

No		IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm), MS *M/Z* (% relative abundance)
9	IR	1681(CO ring), 1732(CO ester), 2994(CH-aliphatic), 3118(CH-aromatic).
	^{1}H-NMR	1.19(t, 3H, CH_2-$\underline{CH_3}$), 4.15(q, 2H, $\underline{CH_2}$-CH_3), 5.15(s, 2H, -N-CH_2-C=O), 7.52-8.26(m, 4H, ArH), 9.94(s, 1H, triazole ring).
	MS	272(M+1, 24.43%), 90(100 %)
10	^{1}H-NMR	1.22(t, 3H, CH_2-$\underline{CH_3}$), 3.3(s, 3H, CH_3 at position 1 of triazole ring), 4.2(q, 2H, $\underline{CH_2}$-CH_3), 5.15(s, 2H, -N-CH_2-C=O), 7.44-8.18(m, 4H, ArH).
12a	^{1}H-NMR	5.15(s, 2H, -N-CH_2-C=O), 7.04-7.59(m, 3H, ArH), 8.23(d,1H, ArH), 9.98(s,1H, position 1 of triazole ring), 10.31(s, 1H, -CO -$\underline{NH}$-Ph).
12b	IR	755(C-Cl), 1681(CO), 2957(CH-aliphatic), 3109(CH-aromatic), 3245(-NH-).
	^{1}H-NMR	5.23(s, 2H, -N-CH_2-C=O), 7.19-7.68(m, 7H, ArH), 8.29(d,1H, ArH), 9.98(s,1H, position 1 of triazole ring), 10.00(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	353(M, 10.32%), 354(M+1, 10.77%), 227(100 % base), 199(92.63%).
12c	IR	752(C-Cl), 1688(CO), 3126(CH-aromatic), 3448(-NH-).
	^{1}H-NMR	5.15(s, 2H, -N-CH_2-C=O), 7.26-7.63(m, 7H, ArH), 8.28(d,1H, ArH), 9.98(s,1H, position 1 of triazole ring), 10.46(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	353(M, 9.87%), 355(M+2, 6.14%), 227(100 % base), 199(96.58%).
12e	IR	1338,1566(*sym*. and *asym*. stretching of NO_2), 1680(CO), 3127(CH-aromatic), 3460(-NH-).
	^{1}H-NMR	5.23(s, 2H, -N-CH_2-C=O), 7.26-7.62(m, 3H, ArH), 7.79-7.87(m, 2H, ArH), 8.21-8.32(m,3H, ArH), 9.99(s,1H, position 1 of triazole ring), 10.98(s, 1H, -CO -$\underline{NH}$-Ph).
12f	IR	1675(CO), 3100(CH-aromatic), 3213(*m, broad*-OH), 3592(*w* -NH-).
	^{1}H-NMR	5.14(s, 2H, -N-CH_2-C=O), 6.43(d, 1H, ArH), 6.90-7.52(m, 6H, ArH), 8.27(d,1H, ArH), 9.45(S, 1H, m-OH of the acetanilide group), 9.97(s,1H, position 1 of triazole ring), 10.24(s, 1H, -CO -$\underline{NH}$-Ph).
12g	IR	1682(CO), 2972(CH-aliphatic), 3108(CH-aromatic), 3450(-NH-).
	^{1}H-NMR	3.71(s,3H, -OCH_3), 5.51(s, 2H, -N-CH_2-C=O), 6.66(d, 1H, ArH), 7.56-7.07(m, 6H, ArH), 8.29(d, 1H, ArH), 9.98(s, 1H, at position 1 of the triazole ring), 10.33(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	349(M^+, 21.33%), 350(M+1, 5.01%), 227(38.48%), 199(64.0%), 123(100 % base).
12h	IR	1690(CO), 2991(CH-aliphatic), 3114(CH-aromatic), 3285(NH).
	^{1}H-NMR	1.3(t,3H, -$OCH_2\underline{CH_3}$), 4.28(q,2H, -$O\underline{CH_2}CH_3$), 5. 2(s, 2H, -N-CH_2-C=O), 7.42-7.61(m, 3H, ArH), 7.72(d, 2H, ArH), 7.93 (d, 2H, ArH), 8.3(d, 1H, ArH), 9.98(s,1H, position 1 of triazole ring), 10.7(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	391(M^+, 2.95%), 199(0.16%), 119(20.75.0%), 92(34.52 %), 91(38.29 %), 54(100% base).
13a	IR	1681(CO), 2954(CH-aliphatic), 3084(CH-aromatic), 3526(-NH-).
	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 5.15(s, 2H, -N-CH_2-C=O), 7.07(t, 1H, ArH), 7.29-7.57(m, 7H, ArH,), 8.17(d, 1H, ArH), 10.34(S,1H, -CO-NH-Ph).
	MS	333(M, 2.3%), 241(100 % base), 213(87.95%).
13b	IR	754(C-Cl), 1675(CO), 3043,3114(CH-aromatic), 3227(-NH-).

	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 5.23(s, 2H, -N-CH_2-C=O), 7.21-7.64(m, 7H, ArH,), 8.17(d, 1H, ArH), 10.00(S,1H, -CO-NH-Ph).
	MS	367(M,10.4%), 368(M+2, 2.4%), 242(34.02%), 213(34.27%), 91(100%base).
13c	IR	749(C-Cl), 1662, 1689(CO),2928(CH-aliphatic), 3118(CH-aromatic), 3323(-NH-).
	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 5.15(s, 2H, -N-CH_2-C=O), 7.35-7.6(m, 7H, ArH,), 8.17(d, 1H, ArH), 10.48(S,1H, -CO-NH-Ph).
	MS	367(M,6.2%), 368(M+2,2.4%), 241(100 % base), 213(71.85%), 116(90.9%).
13d	IR	1340,15139(*sym*. and *asym*. stretching of -NO_2), 1688(CO), 2961(CH-aliphatic), 3111(CH-aromatic), 3339(-NH-).
	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 5.21(s, 2H, -N-CH_2-C=O), 7.37-7.79(m, 6H, ArH,), 7.95(d, 1H, ArH), 8.17(d, 1H, ArH), 10.64(S,1H, -CO-NH-Ph).
13f	IR	1670(CO), 3109(CH-aromatic), 3351(-OH), 3424(-NH-).
	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 5.12(s, 2H, -N-CH_2-C=O), 6.47(d, 1H, ArH), 6.92-7.1(m, 3H, ArH,), 7.39-7.55(m, 3H, ArH,), 8.17(d, 1H, ArH), 9.38(s, 1H, m-OH of the acetanilide group), 10.21(S,1H, -CO-NH-Ph).
13g	IR	1683(CO), 2971(CH-aliphatic), 3144(CH-aromatic), 3311(-NH-).
	^{1}H-NMR	3.04(s, 3H, CH_3 at position 1 of triazole ring), 3.07(s, 3H, -OCH_3), 5.15(s, 2H, -N-CH_2-C=O), 6.65(d, 1H, ArH), 7.07-7.55(m, 6H, ArH,), 8.17(d, 1H, ArH), 10.35(S,1H, -CO-NH-Ph).
13h	^{1}H-NMR	1.3(t, 3H, -$OCH_2\underline{CH_3}$), 2.48(s, 3H, CH_3 in position 1of the triazole ring, overlaped with DMSO peak), 4.29(q, 2H, -$O\underline{CH_2}CH_3$), 5.24(s, 2H, -N-CH_2-C=O), 7.69-7.95(m, 8H, ArH), 10.7(s, 1H, -CO -$\underline{NH}$-Ph).
13i	^{1}H-NMR	3.3(s, 3H, CH_3 in position 1of the triazole ring, coincided with DMSO peak), 5.24(s, 2H, -N-CH_2-C=O), 7.69-7.95(m, 8H, ArH), 10.7(s, 1H, -CO -$\underline{NH}$-Ph).
14a	^{1}H-NMR	5.2(s, 2H, -N-CH_2-C=O), 7.06-7.69(m, 8H, ArH), 7.93(d, 1H, ArH), 10.32(s, 1H, -CO -$\underline{NH}$-Ph).
14b	IR	1168(CO), 3263(CH-aromatic), 3470(-NH-).
	^{1}H-NMR	5.28(s, 2H, -N-CH_2-C=O), 7.2-7.38(m, 2H, ArH), 7.5-7.38(m, 5H, ArH), 7.93(d, 1H, ArH), 9.97(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	421(M, 5.03%), 239(61.96%), 127(100 % base).
14c	IR	1667(CO), 3194(CH-aromatic), 3320(-NH-).
	^{1}H-NMR	5.2(s, 2H, -N-CH_2-C=O), 7.18-7.69(m, 7H, ArH), 7.93(d, 1H, ArH), 10.48(s, 1H, -CO -$\underline{NH}$-Ph).
14d	IR	1352&1510(*sym*. and *asym*. stretching of -NO_2), 1694(CO), 2990(CH-aliphatic), 3093(CH-aromatic), 3297(-NH-).
14e	IR	1342, 1509(*sym*. and *asym*. stretching of -NO_2), 1692(CO), 2968(CH-aliphatic), 3157(CH-aromatic), 3480(-NH-).
	^{1}H-NMR	5.28(s, 2H, -N-CH_2-C=O), 7.29-7.95(m, 6H, ArH), 8.23(d, 2H, ArH), 11.02(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	432(M, 0.01%), 295(100 % base), 267(94.15%).
14f	IR	1667(CO), 3152(CH-aromatic), 3318(b, -OH and -NH overlapped).
	^{1}H-NMR	5.18(s, 2H, -N-CH_2-C=O), 6.49(d, 1H, ArH), 6.5-7.68(m, 6H, ArH), 7.93(d, 1H, ArH), 9.39(S, 1H, m-OH of the acetanilide group),10.18(s, 1H, -CO -$\underline{NH}$-Ph).
14g	IR	1689(CO), 2847, 2966(CH-aliphatic), 3090, 3150(CH-aromatic), 3314(-NH-).
	^{1}H-NMR	3.67(s, 3H, -OCH_3), 5.16(s, 2H, -N-CH_2-C=O), 6.62-7.91(m, 8H, ArH), 10.36(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	417(M^+, 3.45%), 289(100%, base), 274(93.76%), 238(66.31%), 112(23.96).
14h	IR	1602(C=C), 1694(CO), 2998(CH-aliphatic), 3196(CH-aromatic), 3458(-NH-).
	^{1}H-NMR	1.3(t,3H, -$OCH_2\underline{CH_3}$), 4.28(q, 2H, -$O\underline{CH_2}CH_3$), 5.12(s, 2H, -N-CH_2-C=O), 7.28-7.95(m, 8H, ArH), 10.74(s, 1H, -CO -$\underline{NH}$-Ph).
	MS	459(M, 3.93%), 267 (100 % base), 239(40.15%), 165(46.18%), 119(61.75%).

Antimicrobial activity

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

1. *Streptococcus pneumonia* (RCMB 010010) (Gram positive bacteria)
2. *Bacillus subtilis* (RCMB 010067) (Gram positive spore-forming bacteria)
3. *Escherichia coli* (RCMB 010052) (Gram negative bacteria)
4. *Pseudomonas aerugenosa* (RCMB 010043) (Gram negative bacteria)
5. *Aspergillus fumigatus* (RCMB 02568) (a representative of fungi).
6. *Syncephalastrum racemosum* (RCMB 05922) (a representative of fungi).
7. *Geotricum candidum* (RCMB 05097) (a representative of fungi).
8. *Candida albicans* (RCMB 05036) (a representative of fungi).

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 100µl of a solution of the tested compound containing 2mg/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 ºC for 48 hours and the mean zone of inhibition ± standard deviation beyond well diameter were measured in mm. Discs impregnated with DMF were used as control. The antibacterial reference Ampiciliin and Gentamicin and the antifungal reference Amphotericin B discs were tested concurrently as a standard.

Table 4: Results of antimicrobial activity

Comp.	***Fungi***				***Gram Positive Bacteria***		***Gram Negative Bacteria***	
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***
9	15.4± 0.19	14.2± 0.44	15.9± 0.25	NA	14.4± 0.44	16.8± 0.25	NA	11.2± 0.33
12a	20.1± 1.5	20.9± 2.1	21.4± 0.58	NA	18.3± 0.37	21.4± 0.63	NA	20.3± 1.5
12c	16.2± 0.63	20.4± 1.2	22.3± 0.58	NA	19.4± 1.2	21.3± 0.58	NA	20.6± 0.58
12h	18.7± 0.36	16.9± 0.44	20.8± 0.65	NA	19.2± 0.27	20.0± 0.58	NA	16.4± 0.19
13a	21.3± 1.2	22.4± 1.2	23.4± 0.58	NA	21.6± 1.5	25.4± 0.37	NA	23.4± 1.5
13b	14.6± 0.58	12.8± 0.19	15.9± 0.44	NA	16.3± 0.58	17.2± 0.19	NA	12.7± 0.53
13c	17.3± 1.2	13.2± 0.58	15.4± 0.58	NA	18.2± 1.2	20.3± 0.58	NA	15.7±1.5
14a	18.3± 0.58	20.2± 1.5	20.9± 0.58	NA	21.3± 1.2	23.2± 0.58	NA	22.1± 0.63
14c	15.3± 1.2	16.2± 0.58	18.4± 1.2	NA	22.4± 0.44	25.3± 1.5	NA	24.1±2.1
14g	20.7± 0.63	17.0± 0.37	24.2± 0.35	NA	20.0± 0.34	22.1± 0.19	NA	18.7± 0.19
Standerd	***Amphotericin B***				***Ampicillin***		***Gentamicin***	
	23.7± 1.2	19.7± 1.5	28.7± 2.1	25.4± 0.58	23.8±1.2	32.4± 2.1	**17.3± 1.5**	19.9± 0.58

1= *Aspergillus fumigatus* (RCMB 02568) **2** =*Syncephalastrum racemosum* (RCMB 05922)
3= *Geotricum candidum (RCMB 05097)* **4** = *Candida albicans (RCMB 05036)*
5= *Streptococcus pneumonia (RCMB 010010)* **6** = *Bacillus subtilis (RCMB 010067)*
7= *Pseudomonas aerugenosa (RCMB 010043)* **8** = *Escherichia coli (RCMB 010052)*

- RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organism.
- NA: No activity.

Results of antimicrobial activity

From table 4, it was found that *in vitro* antimicrobial testing revealed that compounds **12_a, 12_c, 13_a** and **14a** were found to be more active than the reference drugs amphotericin B and gentamicin on *Syncephalastrum racemosum* and *Escherichia coli*. compound 14c showed the most activity against *Escherichia coli*. Compound **14_g** which bearing trifluoromethyl group in position 1 and an electron donating (methoxy) group in position 3 of phenyl ring in the phenylacetamide residue has broad spectrum antimicrobial activity against the tested organisms. All the tested compounds showed no activity against *Candida albicans* and *Pseudomonas aerugenosa*. The presence of trifluoromethyl group in position 1 has profound effect on biological activity as noticed in compound **14_a, 14_b** and **14_g**.

REFERENCES

A. Iwashita, K. Hattori, H. Yamamoto, J. Ishida, Y. Kido, K. Kamijo, K. Murano, H. Miyake, T. Kinoshita, M. Warizaya, M. Ohkubo, N. Matsuoka, S. Mutoh, (2005) Discovery of quinazolinone and quinoxaline derivatives as potent and selective poly(ADP-ribose) polymerase-1/2 inhibitors, *FEBS Lett.* **579** 1389-1393.

Bharat K. Trivedi and Robert F. Brund, (1988) *J. Med. Chem.* **31**, **,** 1011-1014.

C. Perez-Melero, A.B. Maya, S.B. del Rey, R. Palaez, E. Aballero, M. Medarde, (2004) A new family of quinoline and quinoxaline analogues of combretastatins, *Bioorg. Med. Chem. Lett.* **14** 3771-3774.

Collins C. H. (1964), *Microbiological Methods, Butterworths*, London, pp. 92,

D. Catarzi, V. Colotta, F. Varano, G. Filacchioni, C. Martini, L. Trincavelli, A. Lucacchini(2004), 1,2,4-Triazolo[1,5-*a*]quinoxaline derivatives: synthesis and biological evaluation as adenosine receptor antagonists**,** *ILFarmaco* **59** 71-81.

D. R. Romer, (2009) "Synthesis of 2,3-dichloroquinoxalines via vilsmeier reagent chlorination," *J. Heterocyclic Chem.*, **46**, 2, 317–319.

G. Arthur, K.B. Elor, G.S. Robert, Z.Z. Guo, J.P. Richard, D.R. Stanley, K. John, T.J. Sean, (2005) Novel Transient Receptor Potential Vanilloid 1 Receptor Antagonists for the Treatment of Pain: Structure−Activity Relationships for Ureas with Quinoline, Isoquinoline, Quinazoline, Phthalazine, Quinoxaline, and Cinnoline Moieties, *J.Med. Chem.* **48** 744-752.

G. Sakata, K. Makino, Y. Kurasawa, (1988) Recent Progress in the Quinoline Chemistry. Synthesis and Biological Activity, *Heterocycles* **27** 2481-2515.

H.U. Gali-Muhtasib, M. Diab-Assaf, M.J. Haddadin (2005), Quinoxaline 1,4-dioxides induce G_2/M cell cycle arrest and apoptosis in human colon cancer cells, *Cancer Chemother. Pharmacol.* **55** 369-378.

K. Toshima, T. Ozawa, T. Kimura, S. Matsumara, (2004) The significant effect of the carbohydrate structures on the DNA photocleavage of the quinoxaline–carbohydrate hybrids, *Bioorg. Med. Chem. Lett.* **14** 2777-2779.

L.E. Seitz, W.J. Suling, R.C. Reunolds, (2002) Synthesis and Antimycobacterial Activity of Pyrazine and Quinoxaline Derivatives, *J. Med. Chem.* **45** 5604-5606.

M. K. El-Said, S. M. E. Aly, F. A. Romeih, F. F. Barsoum (1991), and A.B. Hassan, "Synthesis and pharmacological testing of some new phenothiazine derivatives," *Egy. J. Pharm. Sci.*, **32**, , 251–261.

M. M. Badran, K. A. M. Abouzid, and M. H. M. Hussein, (2003) Synthesis of certain substituted quinoxalines as antimicrobial agents (part II), *Arch. Pharm. Res*., **26** 107.

M.R. Myers, W. He, B. Hanney, N. Setzer, M.P. Maguire, A. Zulli, G. Bilder, H. Galzcinski, D. Amin, S. Needle, A.P. Spada, (2003) Potent quinoxaline-Based inhibitors of PDGF receptor tyrosine kinase activity. Part 1: SAR Exploration and Effective Bioisosteric Replacement of a phenyl substituent, *Bioorg. Med. Chem. Lett.* **13** 3091-3095.

Newbold, G.T. and Spring, F. S. (1948) , *J. Chem. Soc*., 519.

R. Sarges, H.R. Howard, R.C. Browne, L.A. Label, P.A. (1990) Seymour, 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants, *J. Med. Chem.* **33** 2240-2254.

Rashed N., El-Masry, A., El-Ashry, E.H., Amer, and Zimmer, H. (1990); *J. Hetrocyclic Chem.* , **27**, , 691.

SA Galal, AS Abdelsamie, SM Soliman, J Mortier, G Wolber, MM Ali, (2013) *Eu. j. med. chem.* **69**, , 115-124.

T. Morokata, K. Suzuki, K. Ida, H. Tsuchiyama, J. Ishikawa, T. Yamada, (2004) Effect of a novel interleukin-5 receptor antagonist, YM-90709 (2,3-dimethoxy-6,6-dimethyl-5,6-dihydrobenzo[7,8]indolizino[2,3-*b*]quinoxaline), on antigen-induced airway inflammation in BN rats, *Int. Immunopharmacol.* **4** 873-883.

V.K. Tandon, D.B. Yadav, H.K. Maurya, A.K. Chaturvedi, P.K. Shukla, (2006) Design, synthesis, and biological evaluation of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones and related compounds as antifungal and antibacterial agents, *Bioorg. Med. Chem.* **14** 6120-6126.

Y.B. Kim, Y.H. Kim, J.Y. Park, S.K. Kim, (2004) Synthesis and biological activity of new quinoxaline antibiotics of echinomycin analogues, *Bioorg. Med. Chem. Lett.* **14** 541-544.

Z. Szekelyhidi, J. Pato, F.Waczek, P. Banhegyi, B. Hegymegi-Barakonyi, D. Eros, G. Meszaros, F. Hollosy, D. Hafenbradl, S. Obert, B. Klebl, G. Keri, L.

Orfi (2005), Synthesis of selective SRPK-1 inhibitors: Novel tricyclic quinoxaline derivatives, *Bioorg. Med. Chem. Lett.* **15** 3241-3246.

تصميم وتشييد بعض مشتقات [4,2,1]ترايازولو[*a*-3,4] كينوكساللينون الجديدة وتقييم نشاطها كمضادات ميكروبيه

للدكتور

عـادل حمـــــدي غيـاتي

مـــــــــــن

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في هذا البحث تم تشييد بعض المشتقات الجديدة من نواة الترايازولوكينوكساللينون وقد تم إثباتها بطرق التحاليل المختلفة كنقطة الانصهار والأشعة تحت الحمراء والرنين النووي المغناطيسي ومطياف الكتلة والتحليل الدقيق للعناصر (الكربون والهيدروجين والنيتروجين) بهدف دراسة تأثيرها كمضادات ميكروبية وذلك باستخدام الأمفوتريسين ــ ب والأمبسلين والجنتاميسين كعقاقير مرجعية (للفطريات ــ البكتريا موجبة الجرام ــ البكتريا سالبة الجرام) وقد وجد للبعض من هذه المواد الجديدة تأثير مماثل للعقار المرجعي في هذا الصدد .