Received: 28 May 2019 | Revised: 21 July 2019 | Accepted: 16 August 2019

DOI: 10.1002/jhet.3716

ARTICLE

WILEY

Exploration of quinoxaline derivatives as antimicrobial and anticancer agents

Ashraf H. Bayoumi[1] | **Adel H. Ghiaty**[1] | **Shimaa M. Abd El-Gilil**[2] | **Ebtehal M. Husseiny**[2] | **Maha A. Ebrahim**[2]

[1] Pharmaceutical Organic Chemistry Department Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt

[2] Pharmaceutical Organic Chemistry Department Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt

Correspondence
Maha A. Ebrahim, Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
Email: maha.ahmed@azhar.edu.eg

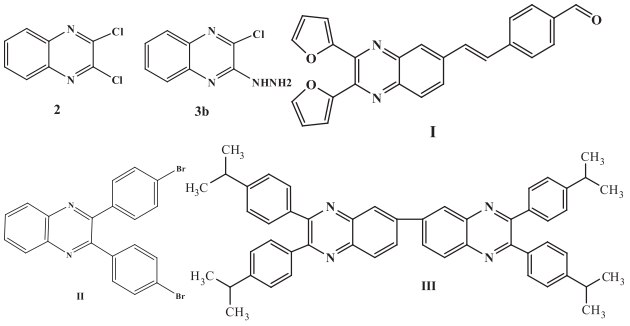
Abstract

Novel 2 and 3-substituted quinoxaline derivatives were synthesized through various synthetic pathways, among which cyanoacetamide and cyanoacetohydrazide quinoxaline derivatives **4a-c** and **11a-c**, respectively, were synthesized. Furthermore, methoxy quinoxaline derivatives **3c** and quinoxaline derivatives bearing substituted pyridines **6a,b**, **12a,b**, and **13a,b** were designed to be synthesized. However, we have synthesized acrylohydrazide **5a,b** and **7**/acrylamide derivatives, Schiff base analogues **14a-f**, pyrazole derivatives **15a-e,** amide derivatives **16a-f**, guanidine derivatives **16 g,h** as well as, quinoxalin-2-methylallyl propionate derivative **14g**. All the synthesized compounds were confirmed *via* spectral data and elemental analyses. Moreover, the newly synthesized compounds were evaluated for their antimicrobial activity (Gm +ve, Gm −ve in comparison to Gentamycin a standard) and fungi (in comparison to Ketoconazole as a standard). Thus, compound **16b** showed promising antimicrobial activity against *B. subtilis*, *P. vulgaris*, and *S. mutants* with values ranging from 20 to 27-mm zone of inhibition. While compounds **5a**, **14e,f,** and **16a,c,d,g,h** showed potent antimicrobial activity. Moreover, the National Cancer Institute (NCI) selected 20 compounds that were submitted for anticancer screening against 60 types of cancer cell lines. The most active compounds are **5b** and **12a** where compound **5b** containing 2,4-dichlorophenyl moiety at cyanoacetamide linkage of hydrazine quinoxaline backbone exerted significant growth inhibition activity against Leukemia MOLT-4, Renal cancer UO-31, and Breast cancer MCF-7. In addition, compound **12a** having 4,6-diaminopyridinone side chain at position-3 of quinoxaline nucleus exhibited remarkable anticancer activity against renal cancer UO-31.

1 | INTRODUCTION

In medicinal chemistry, there has been a lot of interest in quinoxaline as an important class of nitrogen containing heterocyclic compounds. Quinoxaline is a bioactive precursor regarding its diverse biological activities such as: antiinflammatory,[1] antimicrobial,[1,2] antitumor,[3] antiviral[4] antioxidant,[5] antithrombotic,[5,6] and anticonvulsant[6] activities. Considering the significant applications in the field of medicinal chemistry, it was intended to synthesize different derivatives of quinoxaline attached to different substituents and heterocyclic structures at position two in which 3-hydrazinyl and 3-amino quinoxaline derivatives were good scaffolds for these

syntheses. So, it was aimed to synthesize new different quinoxaline derivatives with antimicrobial and anticancer activities. It was reported that 2,3-dichloroquinoxaline **2** and (3-chloro-quinoxalin-2-yl)-hydrazine **3b** have significant antibacterial activity against *Staphylococcus aureus* ATCC 25923.[7] Moreover, 2,3-disubstituted quinoxaline derivatives as compound **I**[7] and **II**[7] showed significant antibacterial activity against *Escherichia coli* and antifungal activity against *Candida albicans*, respectively. Quinoxalines being containing nitrogen atom also reported as a highly selective ATP competitive or mixed competitive for substrate so it is considered an excellent tyrosine kinase inhibitors for different cancer types as chronic myelogenous leukemia.[8] Moreover, compound **III** exhibited excellent anticancer activity against HT-29 and MCF-7 cell lines.[9]



2 | RESULTS AND DISCUSSION

2.1 | *Chemistry*

1,4-Dihydroquinoxaline-2,3-dione **1,**[5,7,10] 2,3-dichloroquinoxaline **2,**[11] 3-hydrazinylquinoxalin-2(*1H*)-one **3a,**[7] and 2-chloro-3-hydrazinylquinoxaline **3b**[7] were prepared as reported.

The alkyl/aryl ether derivative of quinoxaline was prepared from reaction of chloro derivative with methanol in the presence of sodium metal *via* elimination of HCl molecule.[12] So, 2-hydrazinyl-3-methoxy quinoxaline **3c** was synthesized from heating 2-chloro-3-hydrazinyl quinoxaline **3b** in sodium methoxide solution under reflux. The ^{1}H NMR spectrum of compound **3c** exhibited a singlet signal at δ 3.095 ppm due to methoxy protons. While, cyanoacetamide derivatives were prepared through reaction of amino containing compounds with ethylcyanoacetate *via* elimination of ethanol molecule.[13,14] Thus, compounds **4a-c** were synthesized from the reaction of hydrazinyl quinoxaline derivatives **3a-c** with ethylcyanoacetate. The structure of compounds **4a-c** were evidenced by spectral and elemental data. The IR spectrum of these compounds exhibited absorption bands at 2273 to 2240 cm^{-1} corresponding to cyano function as well as absorption bands at 1670 to 1615 cm^{-1} due to amidic carbonyl group. In addition, the ^{1}H NMR spectra of compounds **4a-c** illustrated singlet at δ 3.16 to 4.10 ppm assigned for the methylene protons beside deuterium oxide exchangeable singlet at δ 9.47 to 11.00 ppm corresponding to the amidic NH proton.

Furthermore, the Knovenagal condensation[12,15] of the cyanoacetohydrazinyl derivatives **4a** and **4c** with substituted benzaldehyde afforded compounds **5a** and **7** showed arylidene CH at δ 8.51 to 8.98 ppm as singlet signals.

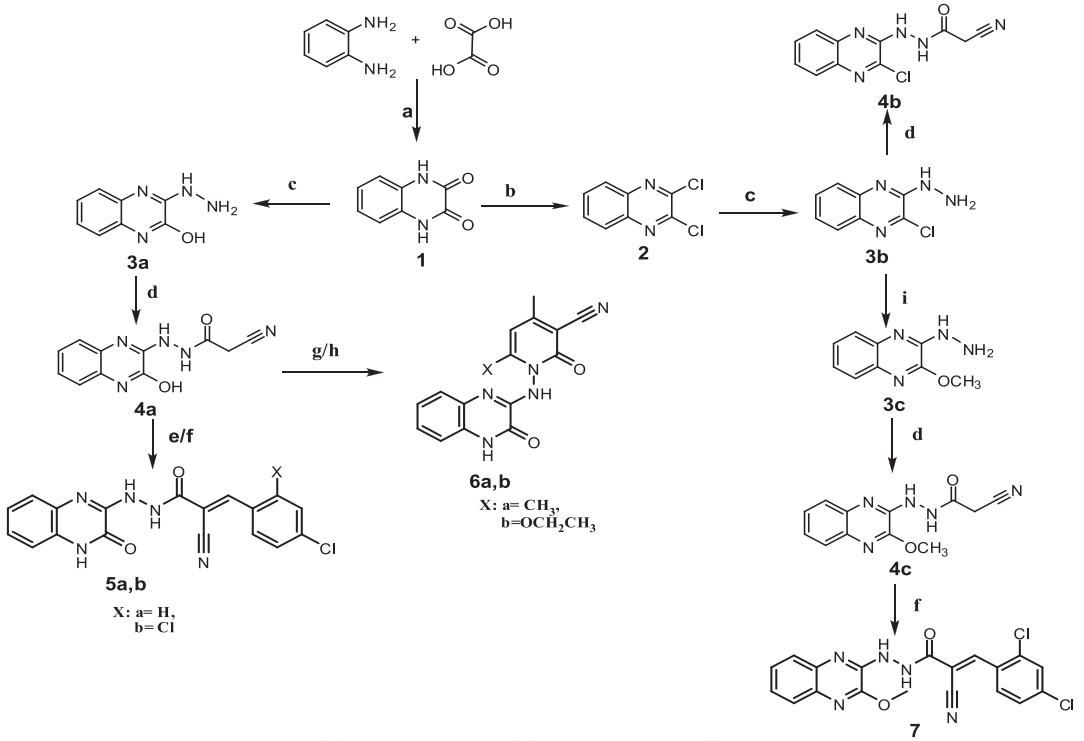
Moreover, cyclocondensation of cyanoacetamide derivatives with β-diketones was previously discussed.[14] So reaction of acetohydrazide derivative **4a** with β-diketones namely, acetyl acetone, and ethylacetoacetate under reflux using a catalytic amount of piperidine afforded the corresponding pyridinone derivatives **6a,b**, respectively.

The ^{1}H NMR spectra of compounds **6a,b** lacked singlet corresponding to methylene protons and amidic NH protons of both compounds and exhibited singlet at δ 5.80 to 6.01 ppm assigned for protons of pyrine C_5 proton.

Also, the ^{13}C NMR spectrum of compound **6a** showed two peaks at δ = 15.55 and 18.99 ppm due to two CH_3. In addition, the ^{13}C NMR spectrum of compound **6b** revealed peaks at δ = 11.75 and 19.10 ppm corresponding to two CH_3 (Scheme 1).

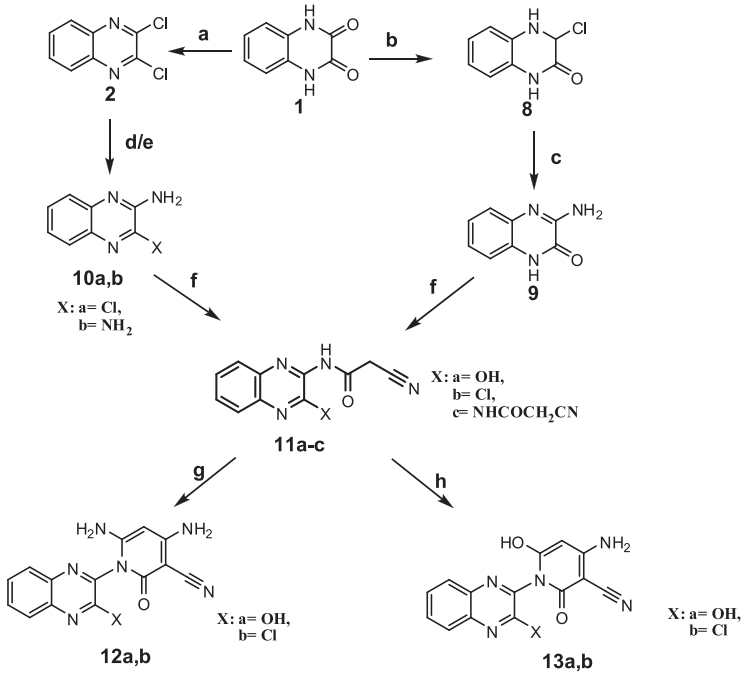
3-Chloroquinoxalin-2(*1H*)-one **8,**[16] 3-aminoquinoxalin- 2(*1H*)-one **9,**[14] 3-chloroquinoxalin-2-amine **10a,**[17] and quinoxaline-2,3-diamine **10b**[18] were prepared as reported. Furthermore, solvent free reaction of compounds **10a,b** with ethylcyanoacetate produced the corresponding cyanoacetamide derivatives **11a,b**. The ^{1}H NMR spectra of compounds **11a-c** revealed singlet signals at δ 4.00 ppm due to CH_2 protons and another deuterium oxide exchangeable singlet signals at δ 11.9 ppm due to two amidic NH protons. The ^{13}C NMR spectrum of compound **11b** showed two peaks at δ 24.18 and 115.58 ppm due to CH_2 and C≡N carbons, respectively. In addition, a peak at δ 167.36 ppm is assigned for amidic carbonyl carbon. 6-Amino-2-oxopyridine-3-carbonitrile derivatives **12a,b** and 6-hydroxy-2-oxopyridine-3-carbonitrile derivatives **13a,b** were obtained from reaction of cyclization of the cyanaoacetamide derivatives **12a,b** with active methylene containing compounds[19] namely, malononitrile, and ethyl cyanoacetate, respectively, under reflux in dioxane containing catalytic amount of piperidine.

The ^{1}H NMR spectra of compounds **12a,b** exhibited D_2O exchangeable singlets at δ 1.54 to 2.00 ppm corresponding to two NH_2 protons as well as a singlet at δ 4.50-4.90 ppm assigned for pyridine CH proton. Also, the ^{13}C NMR spectrum of compound **13a** showed peaks at δ 163.53, 175.44, and 177.91 ppm corresponding to C═O of pyridine, C—OH and C—NH_2, respectively. While, the ^{1}H NMR spectrum of compound **13b** revealed two deuterium oxide exchangeable singlet signals at δ 2.00 and 9.07 ppm due to NH_2 and OH protons, respectively (Scheme 2).



Reagents and conditions: **(a)** H_2O/ conc. HCl/ reflux; **(b)** $SOCl_2$/ CH_2Cl_2/ reflux; **(c)** NH_2NH_2 80%/ absolute ethanol/ reflux; **(d)** $NCCH_2COOC_2H_5$/ absolute ethanol/ reflux; **(e)** 4-Chlorobenzaldehyde/ piperidine/ dioxane/ reflux; **(f)** 2,4-Dichlorobenzaldehyde/ piperidine/ dioxane/ reflux; **(g)** $CH_3COCH_2COCH_3$/ piperidine/ absolute ethanol/DMF/ reflux; **(h)** $CH_3COCH_2COOC_2H_5$/ piperidine/ absolute ethanol/DMF/ reflux.**(i)** Na metal/ methanol/ reflux;

SCHEME 1



Reagents and conditions: **(a)** $SOCl_2/CH_2Cl_2$/reflux; **(b)** $POCl_3$/ CH_2Cl_2/ R.T; **(c)** Conc.NH_3/absolute ethanol/stir, 0°C; **(d)** Conc.NH_3/ DMF/stir, 0°C; **(e)** CH_3COONH_4/ absolute ethanol/ reflux; **(f)** $NCCH_2COOC_2H_5$/ fusion; **(g)** $CH_2(CN)_2$/ piperidine/dioxane; **(h)** $NCCH_2COOC_2H_5$/ piperidine/dioxane.

SCHEME 2

Furthermore, the reaction of hydrazinyl derivatives **3a, b** with different aldehydes, such as 4-hydroxy-benzaldehyde, thiophene-2-carboxaldehyde, and 2-carboxy-benzaldehyde afforded the Schiff bases **14a-f**, respectively. The ^{1}H NMR spectra of compounds **14a-f** revealed singlet signals at δ 7.06 to 9.67 ppm corresponding to N═CH protons. While, ^{13}C NMR of compounds **14a-c** showed signals assigned for N═C carbons at δ 138.17 to 146.52 ppm. It has been noted that 2-benzylidene hydrazinyl-quinoxaline carboxylate derivatives **14e,f** were obtained from the reaction of 2-carboxybenzaldehyde with the hydrazinyl derivatives in methanol where the reaction unexpectedly occurred at aldehydic and not the carboxylic group adopting the reported method.[20]

On the other hand, condensation of hydrazide derivatives with ethyl acetoacetate was previously reported.[21] Thus, hydrazinylidene butanoate derivative **14g** was prepared from the reaction of compound **3b** with ethyl acetoacetate. The structure of compound **14g** was confirmed *via* the presence of triplet and quartet in the ^{1}H NMR spectrum at δ 1.65 and 4.06 ppm characteristic for the ester methyl and the methylene protons. Also, the ^{13}C NMR of compound **14g** revealed signal at δ 159.99 ppm corresponding to N═C carbon. It is well established in the literature that, 3-amino-5-oxopyrazol derivatives were synthesized *via* reaction of different hydrazide derivatives with ethyl cyanoacetate.[21,22] Consequently, reacting the hydrazides **3a**,**b** with ethyl cyanoacetate in sodium methoxide solution afforded the corresponding 3-amino-5-oxo-pyrazole derivatives **15a,b.** The reaction was assumed to occur through nucleophilic addition of NH on cyano function followed by cyclization with removal of ethanol moiety. The ^{1}H NMR spectra of compounds **15a**,**b** showed singlets at δ 2.66 and 2.99 ppm, respectively, characteristic for pyrazole CH_2 protons. While, 3,5-dimethylpyrazole derivatives were reported to be synthesized from the reaction of acid hydrazide derivatives with acetyl acetone.[21,23] Therefore, refluxing hydrazinyl derivatives **3a**,**b** with acetyl acetone yielded the corresponding dimethyl pyrazolyl derivatives **15c,d**. The structure of such compounds was supported by ^{1}H NMR and ^{13}C NMR. In the ^{1}H NMR spectra of compounds **15c,d**, there are singlets at δ 2.19 and 2.04 ppm, respectively, due to pyrazolyl-C_3-CH_3 protons in addition to another singlet at δ 2.30 ppm corresponding to pyrazolyl-C_5-CH_3 protons. Also, the ^{13}C NMR spectra for these compounds showed signals at δ 11.89 to 12.01 ppm and δ 13.73 to 13.81 ppm assigned for pyrazolyl-C_3-CH_3 and pyrazolyl-C_5-CH_3 carbons, respectively.

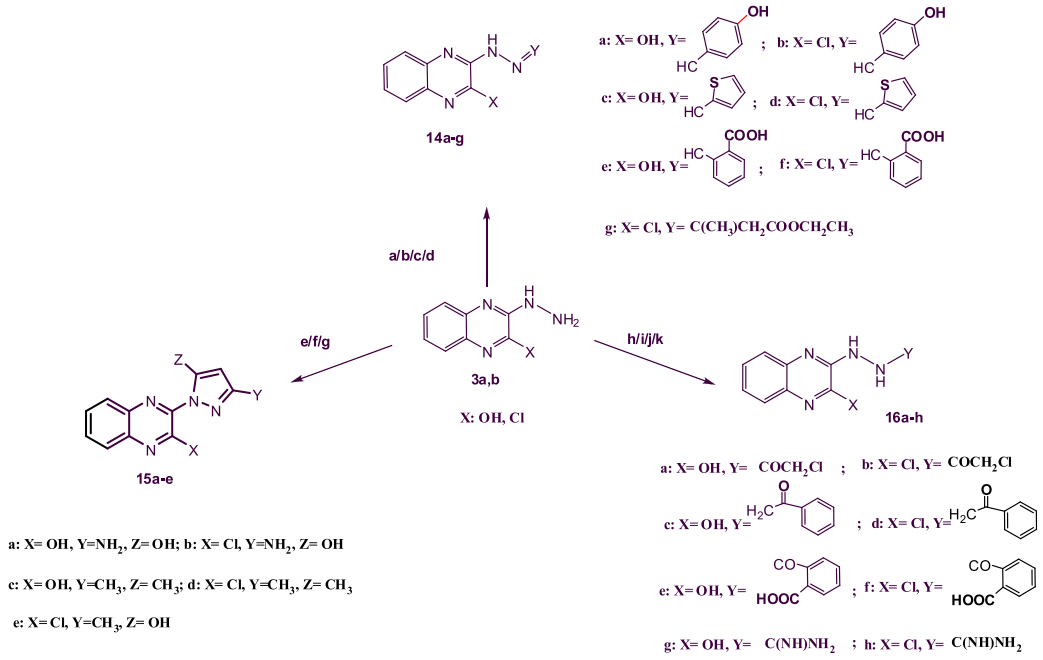
The synthesis of 3-methyl-5-oxo pyrazole derivatives from cyclocondensation of hydrazide derivatives with ethyl acetoacetate has been reported.[21,24] Therefore, compound **3b** reacted with ethyl acetoacetate to yield the corresponding 3-methyl pyrazolone derivative **15e** whose structure was confirmed by the presence of a peak at δ 170.23 ppm in ^{13}C NMR characteristic for carbonyl carbon. A survey in the literature revealed that amino containing compounds reacted with chloroacetyl chloride with elimination of HCl molecule.[25] In this investigation, quinoxaline-2-hydrazide derivatives **3a,b** reacted with chloroacetyl chloride at room temperature in the presence of DMF to afford the corresponding acetohydrazides **16a,b**. The ^{1}H NMR spectra of compounds **16a,b** exhibited singlet signals at δ 5.57 and 5.56 ppm, respectively, corresponding CH_2 protons. While, the ^{13}C NMR spectrum of compound **16b** showed signal at δ 156.75 ppm due to carbonyl carbon. Phenylethanone derivatives were obtained from reaction of phenacyl bromide with the hydrazides **3a,b**. So compounds **3a,b** reacted with phenacyl bromide to yield the corresponding phenylethanone derivatives **16c,d**. The structure of compounds **16c,d** was evidenced by the presence of singlets at δ 4.52 and 4.46 ppm, respectively, characteristic for CH_2 protons. However, nucleophilic addition of hydrazide containing compounds with phthalic anhydride has been reported.[26–28] In this work, compounds **3a,b** reacted with phthalic anhydride in glacial acetic acid to give the corresponding hydrazine carbonyl benzoic acid derivatives **16e,f** and not the isoindoline-1,3-dione derivatives. The ^{13}C NMR spectra of compounds **16e,f** revealed two peaks at δ 165.96 to 166.03 ppm and δ 169.16 to 169.43 ppm assigned for ketonic carbonyl and carboxylic carbonyl functions, respectively.

On the other hand, amino containing compounds reacted with cyanamide to give the corresponding carboximidamide derivatives.[29] Thus, the target compound **16g** was obtained through reaction of hydrazinyl derivative **3a** with cyanamide in acidic medium. The ^{13}C NMR of compound **16g** exhibited signal at δ 163.05 ppm due to C (NH)NH_2 carbon (Scheme 3).

2.2 | Biological evaluation

2.2.1 | *Antimicrobial screening*

Thirty six of the newly synthesized compounds were screened for their antimicrobial activity using the agar diffusion technique. The tested organisms were *S. aureus*, *B. subtilis* (NRRL B-543), and *Streptococcus mutants* (ATCC 25175) as representatives of Gram +ve bacteria, *E. coli* (ATCC 25955), and *Proteus vulgaris* (ATCC 13315) as representatives of Gram −ve bacteria and *C. albicans (ATCC 10231)* as representative of fungi.



Reagents and conditions: **(a)** 4-Hydroxy benzaldehyde/ gl. AcOH/ methanol/ reflux; **(b)** Thiophene-2-carboxaldehyde/ gl. AcOH/ methanol/ reflux; **(c)** 2-Carboxy benzaldehyde/ methanol/ reflux; **(d)** $CH_3COCH_2COOC_2H_5$/ absolute ethanol/ reflux; **(e)** $NCCH_2COOC_2H_5$/ Na metal/ methanol/ Reflux; **(f)** $CH_3COCH_2COCH_3$ absolute ethanol/ reflux; **(g)** $CH_3COCH_2COOC_2H_5$/ fusion; **(h)** $ClCH_2COCl$/ DMF/ stir; **(i)** Phenacyl bromide/ dioxane/ reflux; **(j)** Phthalic anhydride/ gl. ACOH/reflux; **(k)** NH_2CN/ conc.HCl/ absolute ethanol/ reflux.

SCHEME 3 [Color figure can be viewed at wileyonlinelibrary.com] [Color figure can be viewed at wileyonlinelibrary.com]

Agar well diffusion method:

Antimicrobial tests were carried out using the agar well diffusion method on a sterile Petri dish containing Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi; 100 μL of the tested compound solution was prepared by dissolving 1 mg of the chemical compound in 1-mL dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 hours at 37°C for bacteria and 48 hours at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Gentamycin (4 μg/mL) for Gram +ve and Gram −ve bacteria while Ketoconazole (100 μg/mL) as antifungal were used as standards. After incubation, antimicrobial activity was evaluated by measuring the zone of inhibition against tested microorganisms. Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm).[30–34] Inhibition zones (I.Z.) in mm diameter for the newly synthesized compounds were represented in Table 1 and Figure 1.

Conclusion

Among the tested compounds, the following 10 compounds **4a**, **14d**,**e**,**f**, **16a**,**b**,**c**,**d**,**g**,**h** were found to be the most active. Among the different functions which exerted potent antimicrobial activity, the cyanoacetohydrazinyl derivative **4a** as well as acetohydrazinyl derivatives **16a**, **b**. Also, compounds containing thiophene methylene hydrazinyl moiety **14d** showed remarkable antimicrobial activity. Furthermore, compounds bearing benzylidine hydrazinyl carboxylic moiety **14e**,**f** possess significant antimicrobial activity. Finally, compounds having phenylethanone hydrazinyl and guanidine hydrazinyl moieties **16c**,**d**,**g**,**h**, respectively, exerted significant antimicrobial activity. All tested compounds have no activity against fungi. In addition, the most active compounds were further evaluated by determining their minimum inhibitory concentration (MIC), and the data were represented in Table 2 and Figure 2.

2.2.2 | *The minimum inhibitory concentration (MIC)*

MIC is the lowest concentration of a compound required to inhibit the visible growth of a microorganism being investigated after approximately 24 hours of incubation in the appropriate growth medium. The MIC of active compounds was studied against three strains of Gm +ve bacteria (namely: *S. aureus*, *B. subtilis*, and *S. mutants*) and two strains of Gm −ve bacteria (namely: *E. coli*, *P. vulgaris*) in addition to fungi (namely: *C. albicans*). The results obtained explained that 3-oxo substituted quinoxaline with cyanoacetohydrazide side chain **4a** exhibited moderate inhibition activity against *S. aureus*, *B. subtilis*, and *P. vulgaris*, but it exerted no activity against *E. coli*. While, 3-hydroxyquinoxaline containing

TABLE 1 *Inhibition zones (I.Z.) in mm diameter for the newly synthesized compounds*

	Gram +Ve Bacteria			Gram −Ve Bacteria		Fungi
Comp. No	*S. aureus*	*B. subtilis*	*S. mutants*	*E. coli*	*P. vulgaris*	*C. albicans*
3c	**11**	**NA**	**NA**	**NA**	**13**	**NA**
4a	14	11	9	**NA**	12	**NA**
4b	**12**	**NA**	**10**	**NA**	**10**	**NA**
4c	**NA**	8	**NA**	**NA**	12	**NA**
5a	10	14	**12**	**NA**	13	**NA**
5b	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
6a	10	**NA**	**NA**	**NA**	**NA**	**NA**
6b	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
7	**NA**	11	**NA**	10	11	**NA**
11a	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
11b	**NA**	**9**	**NA**	10	9	**NA**
11c	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
12a	10	**NA**	**NA**	**NA**	**NA**	**NA**
12b	14	10	**NA**	11	**NA**	**NA**
13a	12	**NA**	**NA**	**9**	10	**NA**
13b	11	**NA**	**NA**	12	**NA**	**NA**
14a	**NA**	12	**NA**	**NA**	**NA**	**NA**
14b	**NA**	**NA**	**NA**	10	**11**	**NA**
14c	**NA**	10	**NA**	**NA**	11	12
14d	**NA**	13	12	**NA**	11	**NA**
14e	11	15	14	16	12	**NA**
14f	**NA**	15	10	12	**18**	**NA**
14 g	**NA**	13	**NA**	8	**12**	**NA**
15a	**10**	12	**NA**	**NA**	**NA**	**NA**
15b	**NA**	11	**NA**	**NA**	**NA**	**NA**
15c	**9**	8	**NA**	**NA**	**NA**	**NA**
15d	**NA**	**NA**	**NA**	9	**NA**	**NA**
15e	10	**NA**	**NA**	**NA**	**10**	**NA**
16a	10	20	18	14	**17**	**NA**
16b	15	27	20	14	**26**	**NA**
16c	9	15	14	9	**16**	**NA**
16d	**NA**	17	15	**NA**	**16**	**NA**
16e	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
16f	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
16 g	12	18	13	12	**22**	**NA**
16 h	**NA**	15	14	16	**15**	**NA**
	Gentamycin					**Ketoco-nazole**
Standard	24	26	20	30	25	20

Bold entries denote the types and names of microorganisms and compounds number.

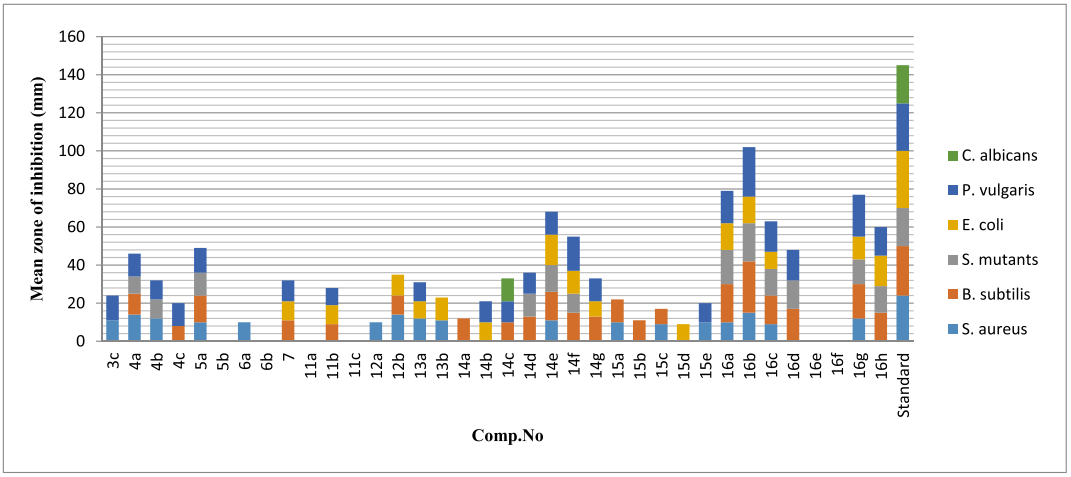


FIGURE 1 Inhibition zones (I.Z.) in mm diameter for the newly synthesized compounds [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Minimum inhibitory concentration (MIC) of the active compounds in μg/mL

Minimum inhibitory concentration in μg/mL						
	Test Organisms					
Comp. No.	Gram +Ve *S. aureus*	*B. subtilis*	*S. mutants*	Gram −Ve *E. coli*	*P. vulgaris*	Fungi *C. albicans*
4a	312.5	625	1250	NA	625	NA
14d	NA	625	1250	NA	312.5	NA
14e	10 000	5000	2500	1125	5000	NA
14f	NA	312.5	625	312.5	156.25	NA
16a	1250	2500	1250	625	625	NA
16b	312.5	19.53	156.25	312.5	19.53	NA
16c	1250	312.5	625	625	312.5	NA
16d	NA	156.25	625	NA	625	NA
16g	1250	2500	1250	1250	39.06	NA
16h	NA	625	10 000	2500	2500	NA

acetohydrazide side chain **16a,b** showed mild inhibition against all tested organisms. Furthermore, hydrazine-1-carboximidamide derivative **16g** showed great antibacterial activity against *P. vulgaris* and mild inhibition activity against: *B. subtilis and E. coli*. Moreover, attachment of hydrazinylidene benzoic acid **14e** at position two of quinoxaline backbone diminished the growth inhibition toward all organisms.

On the other hand, 3-hydrazino substituted quinoxaline derivatives exhibited moderate to promising inhibition especially against *P. vulgaris*. So, compounds containing 2-phenylethanone at hydrazide side chain **16d** exerted moderate inhibition toward all organisms. While, replacement of phenylethanone with chloroacetyl analogue **16b** greatly increases inhibition mainly toward *B. subtilis* and *P. vulgaris*. In addition, replacement of 2-phenylethanone with carboximidamide side chain **16h** highly decreased the growth inhibition of all organisms.

It is to be noted that 3-chloroquinoxaline derivatives having hydrazinylidene linkage at position two **14d** and **16f** showed moderate inhibition toward all organisms.

2.2.3 | *Microplate assay*

The highly active compound **16b** (MIC50 = 4.19 and 4.6 μg/mL for both Gm +ve *B. subtilis* and Gm −ve *P. vulgaris*, respectively) was further submitted to microplate assay[35,36] in comparison to Ciprofloxacin as a reference drug. The experimental results cleared that compound **16b** possesses complete growth inhibition

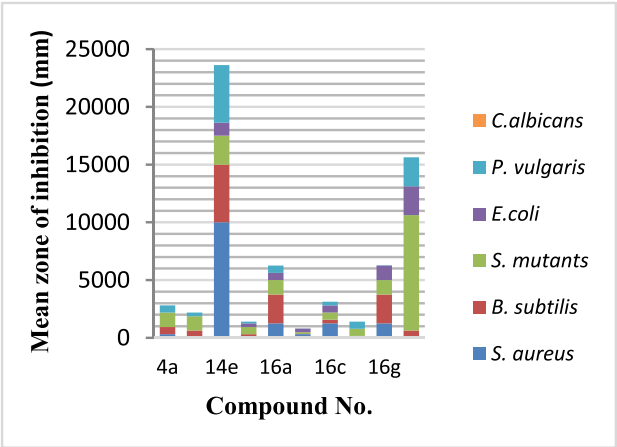


FIGURE 2 Minimum inhibitory concentration (MIC) of the active compounds in μg/mL [Color figure can be viewed at wileyonlinelibrary.com]

activity with concentration range 100 to 25 μg/mL, while excellent growth inhibition at concentration 12.5 μg/mL for both strains (Table 3 and Figure 3).

2.2.4 | *Anticancer screening*

National Cancer Institute (NCI), Bethesda, Maryland, USA has adopted an in vitro model consisting of 60 human tumor cell lines for primary anticancer screening. Screening utilizes 60 different human tumor cell lines representing leukemia, melanoma, and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney.

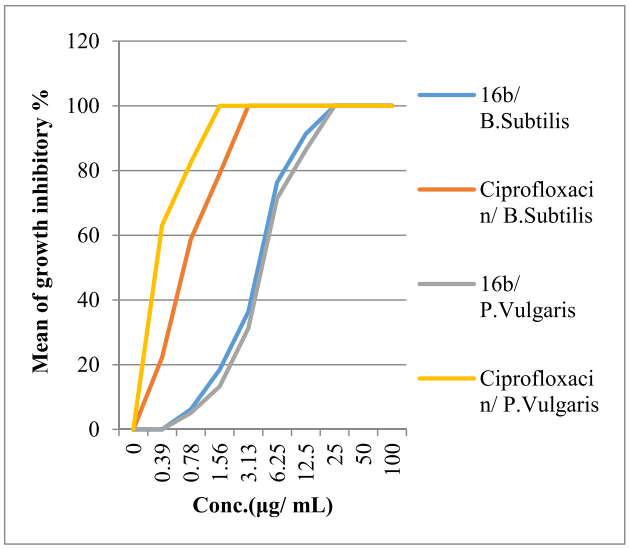


FIGURE 3 Microplate growth inhibition assay of compound 16b [Color figure can be viewed at wileyonlinelibrary.com]

Twenty compounds were evaluated using screening methodology of NCI[37] (Table 4).

The one dose screen results revealed that compound **3c** possessing hydrazinyl moiety at position-3 of quinoxaline backbone illustrated moderate anticancer activity against some cancer cell lines especially CNS cancer SNB-75. While replacement of amino group of hydrazinyl moiety at position-3 and methoxy group at position two with cyanoacetamide function as well as either hydroxyl or chloro group in compounds **4a**,**b**, respectively, resulted in a slight increase in the growth inhibition activity toward many cancer cell lines for example,

TABLE 3 Microplate growth inhibition assay of compound 16b

	Mean of Growth Inhibitory%			
	B. subtilis		*P. vulgaris*	
Conc. μg/mL	**16b**	**Ciprofloxacin**	**16b**	**Ciprofloxacin**
100	100	100	100	100
50	100	100	100	100
25	100	100	100	100
12.5	91.32	100	86.35	100
6.25	76.35	100	71.35	100
3.13	36.37	100	31.25	100
1.56	18.47	78.97	13.25	100
0.78	6.19	58.70	5.09	82.45
0.39	0	22.26	0	63.10
0	0	0	0	0
MIC50	4.19	0.68	4.6	0.29

TABLE 4 Anticancer screening results of selected compounds by NCI

Comp. No.	NSC-No.	Mean Growth %	Delta	Panel	Subpanel Cell Lines (Growth Inhibition %)
3c	813143	26.81	36.86	CNS cancer	SNB-75(26.63).
4a	813147	98.80	25.80	Renal cancer	UO-31 (27).
4b	813142	96.69	26.23	CNS cancer Renal cancer	SNB-75(22.05). A498 (29.54), UO-31(25.06).
5a	813152	98.70	21.41	Leukemia Breast cancer	MOLT-4 (21.83). MCF-7 (22.71).
5b	813153	89.87	29.47	Leukemia Colon cancer CNS cancer Ovarian cancer Renal cancer Breast cancer	MOLT-4 (35.60). EKVX (22.02), NCI-H322M (20.24), NCI-H522 (20.13). SF-268 (24.27), SNB-75 (20.09). OVCAR-4 (26.98). CAKI-1 (27.29), UO31(35.49). MCF-7 (39.60), MDA-MB-231/ATCC (24.40).
6a	813148	95.41	19.23	Non–small cell lung cancer CNS cancer Renal cancer	HOP-92 (23.82). SNB-75 (20.54). UO-31 (21.18).
6b	813141	104.52	17.15	Breast cancer	T-47D (12.63).
7	813146	99.36	21.55	CNS cancer	SNB-75 (22.19).
11a	813145	96.87	20.76	Renal cancer	UO-31 (23.89).
11b	813144	98.16	21.77	Leukemia	SR (23.61).
11c	813156	102.46	20.29	Renal cancer	UO-31 (17.83).
12a	813158	97.18	27.62	Renal cancer	UO-31 (30.44).
12b	813155	106.20	21.43	CNS cancer	SNB-75 (15.23).
13a	813157	104.13	16.99	Renal cancer	UO-31 (12.86).
13b	813154	104.24	21.10	CNS Cancer	SNB-75 (16.86).
14a	813139	104.52	23.34	Renal cancer	UO-31 (18.82).
15a	813150	102.13	22.43	Renal cancer	UO-31 (20.30).
15c	813149	98.84	18.73	Non–small cell lung cancer	HOP-92 (16.34).
15e	813151	104.13	25.03	CNS cancer	SNB-75 (20.90).
16e	813140	94.06	22.85	Leukemia Non–small cell lung cancer CNS cancer Ovarian cancer Breast cancer	CCRF-CEM (22.10), SR (28.79). NCI-H522 (23.64). U251(23.40). OVCAR-4(24.71). MDA-MB-231 (20.72).

renal cancer UO-31 in compound **4a** as well as CNS cancer SNB-75 together with renal cancer A498 and UO-31 in compound **4b**.

Furthermore, compounds **5a**,**b** having chlorophenyl moiety at acetamide linkage exhibited potent anticancer activity against many cancer cell lines. Thus, compound

5a containing 4-chlorophenyl nucleus showed good growth inhibition activity toward leukemia HL-60 (TB) and MOLT-4 in addition to breast cancer MCF-7. However, replacement of hydrogen atom at position-2 of phenyl moiety in compound **5a** by chlorine atom in compound **5b** increased antitumor activity against most cancer cell lines especially leukemia MOLT-4, renal cancer UO-31, and breast cancer MCF-7.

On the other hand, attachment of pyridine ring at hydrazinyl function as in compounds **5a**,**b** decreased the anticancer activity against most cancer cell lines except non–small cell lung cancer HOP-92 and CNS cancer SNB-75 for compound **6a**. Additionally, replacement of oxo function at position two in compound **5b** by methoxy group in compound **7** decreased growth inhibition activity toward many cancer cell lines.

Screening results showed that compounds **11a**-**c** containing cyanoacetamide moiety at position-3 of quinoxaline nucleus displayed significant growth inhibition activity against some cancer cell lines. Thus,

TABLE 5 Physicochemical properties based on Lipiniski's rule of five, TPSA, %ABS, and number of rotatable bonds

	Lipiniski's Parameters							
Compounds	MWt	H-bond donors	H-bond acceptors	MLOGP	Lipiniski violation	TPSA	%ABS	No of Rotatable Bonds
4a	243.22	3	4	0.08	0	110.67	70.82	4
5b	400.22	3	4	2.58	0	110.67	70.82	5
12a	294.27	3	4	0.42	0	143.58	59.46	1
14d	288.76	1	3	2.35	0	78.41	81.95	3
14e	308.29	3	5	1.85	0	107.44	71.93	4
14f	328.74	2	5	2.42	0	87.47	78.82	4
16a	252.66	3	3	0.95	0	86.88	79.03	4
16b	271.10	2	3	1.58	0	66.91	85.92	4
16c	294.31	3	4	1.64	0	86.88	79.03	5
16d	312.75	2	4	2.23	0	66.91	85.92	5
16g	218.22	5	3	0.37	0	119.68	67.71	3
16h	236.66	4	3	1.01	0	99.71	74.60	3

TABLE 6 Pharmacokinetic properties and medicinal chemistry parameters

Compound	GI Absorption	BBB Permeant	P-gp Substrate	Bioavailability Score	CYP1A2	CYP2D6	Log K_p (Skin Permeation), cm/s	Synthetic Accessibility
4a	High	No	No	0.55	No	No	−7.45	2.87
5b	High	No	No	0.55	Yes	No	−6.01	3.38
12a	Low	No	No	0.55	No	No	−7.95	3.08
14d	High	No	No	0.55	Yes	No	−5.13	2.89
14e	High	No	No	0.56	No	No	−6.99	2.91
14f	High	No	No	0.56	No	No	−6.99	2.91
16a	High	No	No	0.55	Yes	No	−6.99	2.89
16b	High	Yes	No	0.55	Yes	No	−6.09	2.78
16c	High	No	No	0.55	Yes	No	−6.11	2.97
16d	High	Yes	No	0.55	Yes	Yes	−5.22	2.80
16g	High	No	No	0.55	Yes	No	−7.56	3.14
16h	High	No	No	0.55	Yes	No	−6.67	3.05

compound **11a** having hydroxyl group at position two showed a reasonable anticancer activity against renal cancer UO-31. However, in compound **11a**, replacement of hydroxyl group by chlorine atom in compound **11b** resulted in a slight decrease in antitumor activity toward most cancer cell lines except for leukemia SR.

Moreover, compound **12a** having 6-aminopyridinone ring at position-3 and hydroxyl group at position-2 of quinoxaline ring showed potent antitumor activity against some cancer cell lines, namely renal cancer UO-31. Although, replacement of hydroxyl group in compounds **12a** by chlorine atom in compound **12b** decreased anticancer activity against most cancer cell lines. Also, substitution of 6-amino pyridinone ring in compounds **12a,b** by 6-hydroxy pyridinone ring in compounds **13a,b** strongly decreased the anticancer activity toward many cancer cell lines.

As indicated from the anticancer results, attachment of *o*-carbonylcarboxylic acid to hydrazinyl group in compound **16e** increased the antitumor activity against most cancer cell lines for example, leukemia CCRF-CEM. It also exerted remarkable anticancer activity toward non-small cell lung cancer NCI-H522, CNS cancer U251, ovarian cancer OVCAR-4, and breast cancer MDA-MB-231/ATCC.

3 | CONCLUSION

It was concluded that, most of compounds exhibited potent anticancer activity against renal cancer UO-31 and CNS cancer SNB-75 cell lines. The most active compounds are **5b** and **12a** where compound **5b** containing 2, 4-dichlorophenyl moiety at cyanoacetohydrazide linkage of quinoxaline backbone exerted significant growth inhibition activity against leukemia MOLT-4, renal cancer UO-31, and breast cancer MCF-7. In addition, compound **12a** having 4,6-diaminopyridinone side chain at position-3 of quinoxaline nucleus showed remarkable anticancer activity against renal cancer UO-31.

In silico studies

The most active antimicrobial and anticancer derivatives were submitted to further in silico studies.

Lipinski's parameters

The most active derivatives were submitted for computational studies of Lipinski's parameters and topological polar surface area (TPSA) were predicted. Lipinski's rule of five is applied to the molecule to predict if it can be used orally or not.[38] All compounds agree with Lipinski's parameters; this indicates that all of them have promising drug-like characters (Table 5).

In silico pharmacokinetic analysis of the most active compounds

Pharmacokinetic properties determine the human therapeutic use of the drugs which include: absorption, distribution, metabolism, excretion, and toxicity (ADMET).[39] So, in silico pharmacokinetic analysis is very important to decrease the percentage drug failure in the step of clinical trials **(**Table 6).

4 | MATERIALS AND METHODS

4.1 | Chemistry

General: Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on KBr plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400-MHz and Mercury 100-MHz NMR spectrometers, Faculty of Pharmacy in Cairo, Bani-Swif, and Mansoura Universities, Egypt in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz). Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the electro-spray (ES) ionization mode. Microanalyses were performed for C, H, and N and were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Center of Mycology and Biotechnology, Al-Azhar University, Egypt. Progress of the reactions was monitored by using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp at 254 nm. Solvent for TLC:dichloromethane:methanol in ratio 9:1 and 8:2.

4.1.1 | 2-Hydrazineyl-3-methoxyquinoxaline 3c

A mixture of chloroquinoxaline **3b** (0.2 g, 1 mmol) and sodium metal (0.23 g, 1.5 mmol) was refluxed in methanol (20 mL) for 12 hours. The reaction mixture was then concentrated, dried, and washed with benzene to give the methoxy derivative **3c**. Gray powder, yield 90%, m.p.: >300°C. IR (KBr, cm^{-1}): 3414 (NH, NH_2); 2988 (CH-aliphatic); 1460 (C═C); 1145 (C—O—C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.04 (s, 2H, NH_2, D_2O exchangeable); 3.10 (s, 3H, CH_3); 3.9 (s, 1H, NH, D_2O exchangeable); 7.71 to 8.10 (m, 4H, quinoxaline-$C_{5,\ 6,\ 7,\ 8}$-H). MS *m/z* (relative intensity %): 190.17 ($M^{+\cdot}$, 0.79); 136 (9.6); 105 (89.87); 77 (100). Anal. form: $C_9H_{10}N_4O$ (190.09). Calcd. (%): C,

56.83; H, 5.30; N, 29.46. Found (%): C, 57.11; H, 5.43; N, 29.73.

4.1.2 | 2-Cyano-N′-(3-substituted quinoxaline-2-yl)acetohydrazide 4a-c

An equimolar mixture of the hydrazinyl derivatives **3a-c** and ethyl cyanoacetate (0.11 g, 0.11 mL, 1 mmol) was heated under reflux in absolute ethanol (15 mL) for 12 hours. The reaction mixture was then concentrated and cooled to room temperature, and the obtained precipitate was collected by suction filtration, dried, and crystallized from dioxane to afford compound **4a,b** and from ethanol to afford **4c**, respectively.

4.1.3 | 2-Cyano-N′-(3-oxo-3,4-dihydroquinoxalin-2-yl)substituted acetohydrazide 4a

Yellow powder, yield: (85%), m.p. > 300°C. IR (KBr, cm^{-1}): 3437 (OH); 3247 (NH); 3050 (CH-aromatic); 2901, 2847 (CH-aliphatic); 2273 (C≡N); 1670 (C═O); 1635 (C═N). ^{1}H NMR (DMSO-d_6) δ: 3.08 (s, 1H, NH—NH—CO, D_2O exchangeable); 3.84 (s, 2H, CH_2); 7.07 to 7.35 (m, 4H, quinoxaline $C_{5,\ 6,\ 7,\ 8}$-H); 8.75 (s, 1H, NH—NH—CO); 12.063 (s, 1H, quinoxaline NH). ^{13}C NMR (DMSO-d_6) δ: 25.10 (CH_2); 114.54 (CN); 123.46, 124.66, 125.43, 128.65, 129.90, 141.66 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 161.68 (quinoxaline C_2); 164.25 (quinoxaline C═O); 169.95 (C═O side chain). MS *m/z* (relative intensity %): 243 ($M^{+\cdot}$, 10.5); 187 (31); 133 (100); 118 (4); 78 (87). . Anal. form: $C_{11}H_9N_5O_2$ (243.08). Calcd. (%): C, 54.32; H, 3.73; N, 28.79. Found (%): C, 54.60; H, 3.81; N, 29.01.

4.1.4 | N′-(3-chloroquinoxalin-2-yl)-2-cyanoacetohydrazide 4b

Dark brown powder, yield: (85%), m.p. 200°C to 201°C. IR (KBr, cm^{-1}): 3290, 3170 (NH), 3051 (CH-aromatic), 2924, 2850 (CH-aliphatic), 2240 (C≡N), 1635 (amidic C═O), 1597 (C═C), 1558 (C═N), 748 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 4.1 (s, 2H, CH_2), 3.98 (s, 1H, NH, D_2O exchangeable), 6.99 to 7.38 (m, 4H, quinoxaline $C_{5,\ 6,\ 7,\ 8}$-H),11.00 (s, 1H, amidic NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 27.13 (CH_2); 115.65 (CN); 121.57, 124.36, 127.26, 129.48, 134.04, 143.23 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 144.70 (C—Cl); 163.83 (quinoxaline C_3); 170.20 (C═O side chain). MS *m/z* (relative intensity %): 263 ($M^{+\cdot}$ + 2, 7), 261 ($M^{+\cdot}$, 10), 146 (53), 117 (90),73 (100). Anal. form: $C_{11}H_8ClN_5O$ (261.04). Calcd. (%): C: 50.49, H: 3.08, N: 26.76. Found (%): C, 50.32; H, 3.17; N, 26.94.

4.1.5 | 2-Cyano-N′-(3-methoxyquinoxalin-2-yl) acetohydrazide 4c

Yellow powder, yield: (69%), m.p. >300°C. (Highly hygroscopic powder). IR (KBr, cm^{-1}): 3219 (2NH), 2976 (CH-aliphatic), 2260 (C≡N), 1615 (C═O); 1264 (C—O). ^{1}H NMR (DMSO-d_6) δ: 3.10 (s, 3H, CH_3), 3.16 (s, 2H, CH_2), 3.95 (s, 1H, NH, D_2O exchangeable), 7.714 to 8.106 (m, 4H, quinoxaline $C_{5,\ 6,\ 7,\ 8}$-H), 9.47 (s, 1H, amidic NH, D_2O exchangeable). MS *m/z* (relative intensity %): 257 ($M^{+\cdot}$, 8), 199 (100), 127 (50), 87 (55.5), 73 (94). Anal. form: $C_{12}H_{11}N_5O_2$ (257.09). Calcd. (%): C, 56.03; H, 4.31; N, 27.22. Found (%): C, 56.12; H, 4.39; N, 27.49.

4.1.6 | 3-(4-Substituted phenyl)-2-cyano-N′-(3-oxo-3,4-dihydroquinoxalin-2-yl) acrylohydrazide 5a,b

An equimolar mixture of the acetohydrazide **4a** (0.24 g, 1 mmol) and substituted benzaldehyde was heated under reflux in dioxane (10 mL) containing few drops of piperidine for 24 hours. The reaction mixture was then concentrated, cooled to afford a precipitate which was collected, dried, and crystallized from ethanol to yield the acrylohydrazide **5a, b**.

4.1.7 | 3-(4-Chlorophenyl)-2-cyano-N′-(3-oxo-3,4-dihydro-quinoxalin-2-yl) acrylohydrazide 5a

Reddish yellow powder, yield: (52%), m.p. 185°C to 186°C. IR (KBr, cm^{-1}): 3440 (OH-tautomer, —NH); 3136 (CH-aromatic); 2278 (C≡N); br. 1664 (2C═O), 1616 (C═N); 813 (1, 4-di-substituted phenyl); 738 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.51(s, 1H, NH—NH—CO, under DMSO, D_2O exchangeable); 7.20 to 7.22 (m, 4H, quinoxaline $C_{5,\ 6,\ 7,\ 8}$-H); 7.52 (d, 2H, J = 8 Hz, 4-chlorophenyl $C_{2,6}$-H); 7.54 (d, 2H, J = 8 Hz, 4-chlorophenyl $C_{3,5}$-H); 8.57 (s, 1H, —C═CH); 11.30 (s, NH—NH—CO, D_2O exchangeable); 12.23 (s, 1H, quinoxaline-NH, D_2O exchangeable). MS *m/z* (relative intensity %): 367 ($M^{+\cdot}$ + 2, 12); 366 ($M^{+\cdot}$ + 1, 17); 363 (26); 319 (100); 204 (45); 125 (61). Anal. form: $C_{18}H_{12}ClN_5O_2$ (365.07). Calcd. (%): C, 59.11; H, 3.31; N, 19.15. Found (%) C, 58.89; H, 3.47; N, 19.43.

4.1.8 | 2-Cyano-3-(2,4-dichlorophenyl)-N′-(3-oxo-3,4-dihydroquinoxalin-2-yl)-acrylohydrazide 5b

Reddish yellow powder, yield: (48%), m.p. 184°C to 185°C. IR (KBr, cm^{-1}): 3445 (OH-tautomer); 3298 (—NH); 3045 (CH-aromatic); 2929 (CH-aliphatic); 2277 (C≡N); br. 1684 (2C═O); 845 (1, 2, 4-trisubstituted phenyl); 757 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.50 (s, 1H, , NH—NH—CO, D_2O exchangeable); 7.19 to 7.25 (m, 4H, quinoxaline $C_{5,\ 6,\ 7,\ 8}$-H); 7.52 (d, 1H, J = 8 Hz, 2, 4-dichloro phenyl-C_6-H); 7.68 (s, 1H, 2, 4-dichloro phenyl-C_3 H); 8.08 (d, 1H, 2, 4-dichloro phenyl-C_5 H); 8.98 (s, 1H, —C═C H); 11.64 (s, 1H, , NH—NH—CO, D_2O exchangeable); 12.44 (s, 1H, quinoxaline NH, D_2O exchangeable). MS m/z (relative intensity %): 403 ($M^{+\cdot}$ + 4, 2); 401 ($M^{+\cdot}$ + 2, 2); 399 ($M^{+\cdot}$, 1.5); 331 (66); 296 (35); 74 (100). Anal. form: $C_{18}H_{11}Cl_2N_5O_2$ (399.03). Calcd. (%): C, 54.02; H, 2.77; N, 17.50. Found (%): C, 54.23; H, 2.94; N, 17.69.

4.1.9 | 4,6-Dimethyl-2-oxo-1-((3-oxo-3,4-dihydroquinoxalin-2-yl)amino)-1,2-dihydropyridine-3-carbonitrile 6a and 6-Ethoxy-4-methyl-2-oxo-1-((3-oxo-3,4-dihydroquinoxalin-2-yl)amino)-1,2-dihydropyridine-3-carbonitrile 6b

An equimolar mixture of the acetohydrazide **4a** (0.24 g, 1 mmol) and acetylacetone/ethyl acetoacetate (1 mmol) was refluxed in ethanol/DMF mixture (5:2, 15 mL) containing piperidine (0.5 mL) for 18 hours. The reaction mixture was then concentrated and cooled at room temperature, and the formed precipitate was collected, dried, and crystallized from DMF to give compounds **6a,b**.

4.1.10 | 4,6-Dimethyl-2-oxo-1-((3-oxo-3,4-dihydroquinoxalin-2-yl)amino)-1,2-dihydropyridine-3-carbonitrile 6a

Yellow powder, yield: (32%), m.p. 190°C to 191°C. IR (KBr, cm^{-1}): 3435 (OH-tautomer); 3170 (NH); 3019 (CH-aromatic); 2850 (CH-aliphatic); 2282 (C≡N); 1671 (C═O), 1616 (C═N); 838 (1, 2, 3, 4-tetra substituted phenyl). ^{1}H NMR (DMSO-d_6) δ: 1.61 (s, 6H, $2CH_3$); 2.50 (s, 1H, hydrazinyl quinoxalinyl-NH, D_2O exchangeable); 6.01 (s, 1H, pyridine- C_5-H); 7.11 to 7.37 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 12.04 (s, 1H, quinoxaline NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 15.55, 18.99 (2 CH_3); 108.99 (pyridine-C_5); 115.82 (pyridine-C_3); 117.67 (CN); 123.29, 124.65, 125.46, 129.95, 132.55, 136.29 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 152.68 (pyridine C_6); 154.56 (pyridine C_4); 159.97 (pyridine C═O); 161.16 (quinoxaline- C_3); 163.88 (quinoxaline C═O). MS m/z (relative intensity %): 307 ($M^{+\cdot}$, 4); 228 (63); 161 (100); 89 (80); 84 (88). Anal. form: $C_{16}H_{13}N_5O_2$ (307.11). Calcd. (%): C, 62.53; H, 4.26; N, 22.79. Found (%): C, 62.84; H, 4.38; N, 23.05.

4.1.11 | 6-Ethoxy-4-methyl-2-oxo-1-((3-oxo-3,4-dihydroquinoxalin-2-yl)amino)-1,2-dihydropyridine-3-carbonitrile 6b

Yellow powder, yield: (32%), m.p. 185°C to 186°C. IR (KBr, cm^{-1}): 3442 (OH-tautomer, —NH); 3198 (CH-aromatic); 2990 (CH-aliphatic); 2280 (C≡N); 1680 (C═O); 1608 (C═N); 848 (1, 2, 3, 4-tetra substituted phenyl). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.61 (t, 3H, —OCH_2CH_3); 2.35 (s, 3H, pyridine C_4-CH_3); 2.47 (s, 1H, hydrazinyl NH, under DMSO, D_2O exchangeable); 3.84 (m, 2H, —OCH_2); 5.80 (s, 1H, pyridine C_5-H); 7.09 to 7.36 (m, 4H, quinoxaline $C_{5,\ 6,7,8}$-H); 12.95 (s, 1H, quinoxaline NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ: 11.75, 19.10 (2 CH_3); 47.67 (CH_2); 100.91 (pyridine- C_5); 114.67 (CN); 123.46, 124.66, 125.34, 129.65, 133.00, 137.29 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 151.68 (pyridine C_6); 152.56 (pyridine C_4); 159.46 (pyridine C═O); 161.68 (quinoxaline-C_3); 164.25 (quinoxaline C═O). MS m/z (relative intensity %): 337 ($M^{+\cdot}$, 14); 314 (36); 215 (83); 187 (39); 105 (67), 50 (100). Anal. form: $C_{17}H_{15}N_5O_3$ (337.12). Calcd. (%): C, 60.53; H, 4.48; N, 20.76. Found (%): C, 60.81; H, 4.59; N, 21.02.

4.1.12 | 2-Cyano-3-(2,4-dichlorophenyl)-N′-(3-methoxyquinoxalin-2-yl)acrylohydrazide 7

An equimolar mixture of compound **4c** (0.26 g, 1 mmol) and 2,4-dichlorobenzaldehyde (0.174 g, 1 mmol) was heated under reflux in dioxane (10 mL) containing piperidine (few drops) for 6 hours. The reaction mixture was then concentrated, cooled, dried, and crystallized from ethanol to produce compound **7**. Yellowish white powder, yield: (47%), m.p. >300°C. IR (KBr, cm^{-1}): 3471 (2NH); 2265 (C≡N); 1633 (C═O); 1464 (C═C); 847 (1, 2, 4- trisubstituted phenyl). ^{1}H NMR (DMSO-d_6) δ: 3.34 (s, 1H, NH—NH-CO, D_2O exchangeable); 3.85 (s, 3H, OCH_3); 7.58 to 7.78 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 7.98 (d, 2H, J = 8 Hz, 2,4-dichlorophenyl $C_{5,6}$-H); 8.06 (s, 1H, 2,4-dichlorophenyl C_3-H); 8.51 (s, 1H, ═CH—); 9.85 (s, 1H, NH—NH—CO, D_2O exchangeable). MS m/z (relative intensity %): 417 ($M^{+\cdot}$ + 4, 16); 415 (58, M + 2); 414 (100, M + 1); 413 (39, M); 348 (94); 231 (77); 119 (83) . Anal. form: $C_{19}H_{13}Cl_2N_5O_2$ (413.04). Calcd. (%): C,

55.09; H, 3.16; N, 16.91. Found (%): C, 55.23; H, 3.40; N, 16.73.

4.1.13 | 2-Cyano-N-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetamide 11a, N-(3-chloroquinoxalin-2-yl)-2-cyanoacetamide 11b and N,N′-(quinoxaline-2,3-diyl)bis(2-cyanoacetamide) 11c

3-Aminoquinoxalin-2(1*H*)-one **9**, 2-amino-3-chloroquinoxaline **10a**, and 2, 3-diaminoquinoxaline **10b** (1 mmol) were fused with excess ethyl cyanoacetate (0.34 g, 0.33 mL, 3 mmol) at 210°C for 30, 1, and 10 hours, respectively. The reaction mixture was then triturated with ethanol, left to evaporate to afford black powder that was washed with diethyl ether on hot to afford cyanoacetamide derivative **11a,** crystallized from methylene chloride/methanol mixture (9.5: 0.5) to afford **11b** and crystallized from DMF to afford **11c**, respectively.

4.1.14 | 2-Cyano-N-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetamide 11a

Brown powder, yield: (37%), m.p. >300°C. IR (KBr, cm^{-1}): 3402 (OH-tautomer); 3046 (CH-aromatic); 2962, 2869 (CH-aliphatic); 2203 (C≡N); 1630 (C═O). ^{1}H NMR (DMSO-d_6) δ: 4.00 (s, 2H, CH_2); 7.07 to 7.14 (m,4H, C_5-C_8-quinoxaline); 11.9 (s, 2H, 2-NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 22.98 (CH_2); 115.58 (CN); 121.46, 124.25, 127.07, 129.44, 132.04, 151.23 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 164.08 (quinoxaline C_3); 166.15 (quinoxaline C═O); 175.93 (amidic C═O).MS *m/z* (relative intensity %): 228 ($M^{+\cdot}$, 28); 220 (35); 134 (33); 106 (62); 79 (100). Anal. form: $C_{11}H_8N_4O_2$ (228.06). Calcd. (%): C, 57.89; H, 3.53; N, 24.55. Found (%): C, 58.04; H, 3.79; N, 24.13.

4.1.15 | N-(3-chloroquinoxalin-2-yl)-2-cyanoacetamide 11b

White powder, yield: (81%), m.p. >300°C. IR (KBr, cm^{-1}): 3434 (OH-tautomer); 3048 (CH-aromatic); 2962, 2876 (CH-aliphatic); 2273 (C≡N);1684 (C═O); 760 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 4.00 (s, 2H, CH_2); 7.07 to 7.35 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 11.92 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 24.18 (CH_2); 115.58 (CN); 122.34, 123.44, 126.04, 128.37, 132.23, 140.46 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 145.07 (C—Cl); 162.20 (quinoxaline C_3); 167.36 (amidic C═O). MS *m/z* (relative intensity %): 248 ($M^{+\cdot}$ +2, 1.23); 246 (M, 9); 185 (11); 64 (100). Anal. form: $C_{11}H_7ClN_4O$ (246.03). Calcd. (%): C, 53.56; H, 2.86; N, 22.71. Found (%): C, 53.73; H, 3.12; N, 22.87.

4.2 | N,N′-(quinoxaline-2,3-diyl)bis(2-cyanoacetamide) 11c

Black powder, Yield 71%, m.p. >300°C. IR (KBr, cm^{-1}): 3435 (NH); 3033 (CH-aromatic); 2800 (CH-aliphatic); 2240 (C≡N); 1668 (C═O); 1612 (C═N); 1408 (C═C). ^{1}H NMR (DMSO-d_6) δ: 4.00 (s, 4H, 2-CH_2); 6.95-7.33 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 11.90 (s, 2H, 2-NH, D_2O exchangeable). MS *m/z* (relative intensity %): 295 ($M^{+\cdot}$ +1, 32); 294 ($M^{+\cdot}$, 41); 249 (43); 175 (57); 121 (82); 65 (100). Anal. form: $C_{14}H_{10}N_6O_2$ (294.09). Calcd. (%): C, 57.14; H, 3.43; N, 28.56. Found (%): C, 57.38; H, 3.67; N, 28.79.

4.3 | 4,6-Diamino-2-oxo-1-(3-oxo-3,4-dihydroquinoxalin-2-yl)-1,2-dihydropyridine-3-carbonitrile 12a and 4,6-Diamino-1-(3-chloroquinoxalin-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 12b

An equimolar mixture of the cyanoacetamide **11a,b** (1 mmol) and malononitrile (0.066 g, 0.1 mL, 1 mmol) was heated under reflux in dioxane (10 mL) containing piperidine (0.5 mL) for 7 to 9 hours. The reaction mixture was then concentrated and cooled, and the precipitate formed was collected, dried, and crystallized from DMF and ethanol to produce compound **12a,b,** respectively.

4.4 | 4,6-Diamino-2-oxo-1-(3-oxo-3,4-dihydroquinoxalin-2-yl)-1,2-dihydropyridine-3-carbonitrile 12a

Dark brown powder, yield: (42%), m.p. >300°C. IR (KBr, cm^{-1}): 3470 (OH-tautomer); 3300, 3250 (NH, NH_2); 3027 (CH-aromatic); 2277 (C≡N); 1670 (C═O); 1600 (C═N); 1434 (C═C). ^{1}H NMR (DMSO-d_6) δ: 2.00 (s, 4H, 2 NH_2, D_2O exchangeable); 4.90 (s, 1H, pyridine C_5-H); 7.07 to 7.14 (m, 4H, quinoxaline, $C_{5,6,7,8}$-H); 11.90 (s, 1H, quinoxaline NH, D_2O exchangeable). MS *m/z* (relative intensity %): 295 ($M^{+\cdot}$ +1, 4); 294 ($M^{+\cdot}$, 13); 161 (46); 106 (100). Anal. form: $C_{14}H_{10}N_6O_2$ (294.09). Calcd. (%): C, 57.14; H, 3.43; N, 28.56. Found (%): C, 57.42; H, 3.60; N, 28.42.

4.5 | 4,6-Diamino-1-(3-chloroquinoxalin-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 12b

White powder, yield: (55%), m.p. >300°C. IR (KBr, cm^{-1}): 3375 (NH_2); 3075 (CH-aromatic); 2153 (C≡N);1666 (C═O); 1593 (C═N); 1455 (C═C); 775 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.54 (s, 2H, pyridine C_6-NH_2, D_2O exchangeable); 1.70 (s, 2H, pyridine C_4-NH_2, D_2O exchangeable); 4.50 (s, 1H, pyridine C_3-H); 7.06 to 7.15 (m, 4H, quinoxaline $C_{5,6,7,8}$-H). MS *m/z* (relative intensity %): 314 ($M^{+\cdot}$ +2, 2); 312 ($M^{+\cdot}$, 4); 77 (9); 70 (100); 54 (49). Anal. form: $C_{14}H_9ClN_6O$ (312.05). Calcd. (%): C, 53.77; H, 2.90; N, 26.87. Found (%): C, 53.89; H, 3.04; N, 27.12.

4.6 | 4-Amino-6-hydroxy-2-oxo-1-(3-substituted-quinoxalin-2-yl)-1,2-dihydropyridine-3-carbonitrile 13a,b

An equimolar mixture of the cyanoacetamide **11a**,**b** (1 mmol), ethyl cyanoacetate (0.11 g, 0.11 mL, 1 mmol) was heated under reflux in dioxane (10 mL) containing piperidine (0.5 mL) for 7 and 24 hours, respectively. The reaction mixture was then concentrated, cooled to afford precipitate that was collected, dried, and crystallized from DMF and methanol to yield compound **13a,b,** respectively.

4.7 | 4-Amino-6-hydroxy-2-oxo-1-(3-oxo-3,4-dihydroquinoxalin-2-yl)-1,2-dihydropyridine-3-carbonitrile 13a

Black powder, yield: (46%), m.p. >300°C. IR (KBr, cm^{-1}): 3438, 3342 (OH-tautomer, NH, NH_2); 3049 (CH-aromatic); 2215 (C≡N); 1685 (C═O); 1476 (C═C). ^{1}H NMR (DMSO-d_6) δ: 2.48 (s, 2H, NH_2, D_2O exchangeable); 3.975 (s, 1H, CH-pyridine); 7.043 to 7.115 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 11.88 (s, 2H, NH and OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 70.15 (pyridine-C_3); 75.80 (pyridine-C_5); 115.58 (CN); 123.46, 126.06, 128.15, 129.88, 143.22, 155.63 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 160.83 (quinoxaline C═O); 162.67 (quinoxaline-C_3); 163.53 (pyridine C═O); 175.44 (C—OH); 177.91 (C—NH_2). MS *m/z* (relative intensity %): 295 ($M^{+\cdot}$, 16); 217 (18); 162 (45); 106 (100). Anal. form: $C_{14}H_9N_5O_3$ (295.07). Calcd. (%): C, 56.95; H, 3.07; N, 23.72. Found (%): C, 56.87; H, 3.29; N, 23.88.

4.8 | 4-Amino-1-(3-chloroquinoxalin-2-yl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile 13b

White powder, yield: (57%), m.p. >300°C. IR (KBr, cm^{-1}): 3394 (OH, NH_2); 3050 (CH-aromatic); 2151 (C≡N); 1658 (C═O); 1592 (C═N); 1454 (C═C); 770 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.00 (s, 2H, NH_2, D_2O exchangeable); 6.00 (s, 1H, pyridine C_5 H); 7.36 to 7.47 (m, 4H, quinoxalin $C_{5,6,7,8}$- H); 9.07 (s, 1H, OH, D_2O exchangeable). MS *m/z* (relative intensity %): 315 ($M^{+\cdot}$ +2, 11); 313 ($M^{+\cdot}$, 13); 229 (40); 139 (7); 119 (100). Anal. form: $C_{14}H_8ClN_5O_2$ (313.04). Calcd. (%): C, 53.60; H, 2.57; N, 22.33. Found (%): C, 53.83; H, 2.69; N, 22.60.

4.9 | 3-(2-(4-Hydroxybenzylidene) hydrazineyl)quinoxalin-2(1H)-one 14a, 4-((2-(3-chloroquinoxalin-2-yl) hydrazineylidene)methyl)phenol 14b, 3-(2-(thiophen-2-ylmethylene)hydrazineyl) quinoxalin-2(1H)-one 14c and 2-chloro-3-(2-(thiophen-2-ylmethylene)hydrazinyl) quinoxaline 14d

To a suspension of the hydrazinyl derivative **3a,b** (1 mmol) in methanol (10 mL) containing glacial acetic acid (4 mL) and dioxane (2 mL), 4-hydroxy benzaldehyde/thiophen-2-carboxaldehyde (1 mmol) were added. The reaction mixture was then heated under reflux for 9 hours, concentrated, allowed to cool to form a precipitate that was crystallized from dioxane to afford the derivatives **14a,c** and from ethanol to afford **14b**,**d**.

4.10 | 3-(2-(4-Hydroxybenzylidene) hydrazineyl)quinoxalin-2(1H)-one 14a

Yellow crystals, yield: (85%), m.p. 251°C to 252°C. IR (KBr, cm^{-1}): 3463 (OH); 3122 (NH); 3012 (CH-aromatic); 2909, 2847 (CH-aliphatic); 1675 (C═O); 1589 (C═N); 829 (1, 4-disubstituted phenyl). ^{1}H NMR (DMSO-d_6) δ: 2.17 (s, 1H, NH, D_2O exchangeable); 6.82 to 6.8 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 7.56 (d, 2H, J = 6 Hz, 4-OH-phenyl-$C_{2,6}$- H); 7.69 (d, 2H, J = 6 Hz, 4-OH-phenyl-$C_{3,5}$ - H); 8.29 (s, 1H, —N═CH); 10.01 (s, 1H, quinoxaline NH, D_2O exchangeable); 11.00 (s, 1H, phenyl OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 111.16, 111.42 (phenyl C_3, C_5); 123.46, 123.95, 124.78, 125.74, 126.21, 145.20 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 129.14, 130.58, 131.00 (phenyl C_1, C_2, C_6); 146.52 (CH=N); 160.17 (phenyl C_4); 160.99 (C_3-quinoxaline); 165.72 (C═O). MS *m/z* (relative intensity %): 280

($M^{+\cdot}$,77); 200 (85); 129 (97); 105 (74); 42 (100). Anal. form: $C_{15}H_{12}N_4O_2$ (280.1). Calcd. (%): C, 64.28; H, 4.32; N, 19.99. Found (%): C, 64.45; H, 4.51; N, 20.08.

4.11 | 4-((2-(3-Chloroquinoxalin-2-yl) hydrazineylidene)methyl) phenol 14b

Red crystals, yield: (95%), m.p. 150°C to 151°C. IR (KBr, cm^{-1}): 3396 (br., OH); 3273 (NH); 3050 (CH-aromatic); 1606 (C═N);1435 (C═C); 827 (1,4-disubstituted-phenyl). ^{1}H NMR (DMSO-d_6) δ: 3.42 (s, 1H, NH, D_2O exchangeable); 6.93 (d, 2H, J = 8HZ, 4-hydroxy phenyl $C_{2,6}$ H); 7.12 (t, 1H, J = 8 Hz, quinoxaline-C_6-H); 7.20 (t, 1H, J = 8 Hz, quinoxaline C_7-H); 7.64 (d, 2H, J = 8 Hz, 4-hydroxy phenyl C_3-H); 7.79 (d, 2H, J = 8 Hz, quinoxaline C_8-H); 9.11 (s, 1H, —N═CH); 10.20 (s, 1H, phenyl OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 115.37, 116.34 (phenyl C_3, C_5); 122.82, 123.03, 125.30, 125.70, 126.64, 128.52 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 129.04, 130.20, (phenyl C_2, C_6); 138.17 (CH═N side chain); 145.01 (C—Cl); 160.71 (C1-phenyl); 162.84 (phenyl C_4); 163.90 (quinoxaline C_3). MS m/z (relative intensity %): 300 ($M^{+\cdot}$ +2, 9); 298 ($M^{+\cdot}$, 15); 276 (100); 219 (48); 115 (86). . Anal. form: $C_{15}H_{11}ClN_4O$ (298.06). Calcd. (%): C, 60.31; H, 3.71; N, 18.76. Found (%): C, 60.49; H, 3.83; N, 19.02.

4.12 | 3-(2-(Thiophen-2-ylmethylene) hydrazineyl)quinoxalin-2(1H)-one 14c

Black crystals, yield: (85%), m.p. 110°C to 111°C. IR (KBr, cm^{-1}): 3456 (OH tautomer and NH); 3198, 3086 (CH-aromatic); 2910, 2846 (CH-aliphatic); 1679 (C═O); 1589 (C═N), 1037 (C—S—C). ^{1}H NMR (DMSO-d_6) δ: 7.13 to 7.22 (m, 4H, quinoxaline $C_{5,6,7,8}$- H); 7.380 (d, 1H, J = 6 Hz, thiophene C_3-H); 7.52 (d, 1H, J = 6 Hz, thiophene C_5-H); 7.647 (t, 1H, J = 6 Hz, thiophene-C_4-H); 8.79 (s, 1H, N═CH); 11.23 (s, 1H, quinoxaline C_3-NH, D_2O exchangeable); 12.39 (s, 1H, quinoxaline-NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 123.08, 123.98, 125.12, 125.95, 126.07, 128.34, 128.77, 128.85, 129.12, 140.04 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a} and thiophene $C_{2,3,4,5}$-H); 142.24 (CH═N); 161.27 (quinoxaline C_3); 162.95 (quinoxaline C═O). MS m/z (relative intensity %): 270 ($M^{+\cdot}$, 13); 268 (100); 206 (33); 104 (68.5); 77 (74). Anal. form: $C_{13}H_{10}N_4OS$ (270.31). Calcd. (%): C, 57.76; H, 3.73; N, 20.73; S, 11.86. Found (%): C, 58.02; H, 3.84; N, 20.97; S, 12.02.

4.13 | 2-Chloro-3-(2-(thiophen-2-ylmethylene)hydrazinyl) quinoxaline 14d

Reddish brown crystals, yield: (98%), m.p. 229°C to 230°C. IR (KBr, cm^{-1}): 3414, 3267 (NH); 3167, 3072 (CH-aromatic); 2969 (CH-aliphatic); 1605, 1553 (C═N); 1420 (C═C); 1031 (C—S—C). ^{1}H NMR (DMSO-d_6) δ: 7.18 to 7.27 (m, 3H, thiophen $C_{3,4,5}$-H); 7.63 to 8.03 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 9.64 (S, 1H, —N═CH); 12.38 (s, 1H, —NH, D_2O exchangeable). MS m/z (relative intensity %): 290 ($M^{+\cdot}$ +2, 6); 288 ($M^{+\cdot}$, 11); 267 (69); 119 (30); 70 (100). Anal. form: $C_{13}H_9ClN_4S$ (288.02). Calcd. (%): C, 54.07; H, 3.14; N, 19.40; S, 11.10. Found (%): C, 54.23; H, 3.40; N, 19.28; S, 11.23.

4.14 | 2-((2-(3-Substituted quinoxalin-2-yl) hydrazinylidene) methyl)benzoic acid 14e,f

An equimolar mixture of the hydrazinyl derivative **3a,b** (1 mmol) and 2-carboxy benzaldehyde (0.15 g, 1 mmol) was heated under reflux in methanol for 10 hours. The reaction mixture was then concentrated, cooled, triturated with ethanol to form a precipitate that was collected, dried, and crystallized from ethanol to yield compound **14e** and from DMF to afford compound **14f**, respectively.

4.15 | 2-((2-(3-Chloroquinoxalin-2-yl) hydrazinylidene)methyl) benzoic acid 14e

Yellow crystals, yield: (59%), m.p. >300°C. IR (KBr, cm^{-1}): 3412 (br, COOH); 3215 (NH); 3072 (CH-aromatic); 2906, 2847 (CH-aliphatic); 1678 (C═O). ^{1}H NMR (DMSO-d_6) δ: 3.1 (s, 1H, NH, D_2O exchangeable); 7.06 to 7.20 (m, 5H, quinoxaline $C_{5,6,7,8}$ and -N═CH); 7.34 (d, 1H, J = 6 Hz, benzoic acid C_6-H); 7.52 (t, 1H, J = 6 Hz, benzoic acid C_5-H); 7.66 (t, 1H, J = 6 Hz, benzoic acid C_4-H); 7.89 (d, 1H, J = 6 Hz, benzoic acid, C_3-H); 12.00 (s, 1H, COOH, D_2O exchangeable); 12.38 (s, 1H, quinoxaline NH, D_2O exchangeable). MS m/z (relative intensity %): 308 ($M^{+\cdot}$, 17.5); 289 (56.8); 160 (5); 104 (100); 76 (50). Anal. form: $C_{16}H_{12}N_4O_3$ (308.29). Calcd. (%): C, 62.33; H, 3.92; N, 18.17. Found (%): C, 62.51; H, 4.08; N, 18.29.

4.16 | 2-((2-(3-Chloroquinoxalin-2-yl) hydrazinylidene)methyl) benzoic acid 14f

Yellowish red powder, yield: (80%), m.p. >300°C. IR (KBr, cm^{-1}): 3400, 3163 (OH, NH); 3020 (CH-aromatic); 2938 (CH-aliphatic); 1675 (C═O); 1500 (C═N); 1471 (C═C). ^{1}H NMR (DMSO-d_6) δ: 3.35 (s, 1H, NH, D_2O exchangeable); 7.12 (t, 1H, J = 8 Hz, quinoxalin C_6-H);

7.13 (d, 1H, $J = 8$ Hz, quinoxaline C_5-H); 7.40 (d, 1H, $J = 8$ Hz, quinoxaline C_8-H); 7.55 (t, 1H, $J = 8$ Hz, quinoxaline C_7-H); 7.57 (t, 1H, $J = 8$ Hz, benzoic acid C_5-H); 7.69 (t, 1H, $J = 8$ Hz, benzoic acid C_4-H); 7.91 (d, 1H, J = 8 Hz, benzoic acid C_6-H); 8.36 (s, 1H, -N═CH); 9.22 (d, 1H, $J = 8$ Hz, benzoic acid C_3-H); 11.15 (s, 1H, -COOH, D_2O exchangeable). MS *m/z* (relative intensity %): 328 ($M^{+\cdot}$ +2, 6); 326 ($M^{+\cdot}$, 14); 272 (100); 257 (39); 75 (65). Anal. form: $C_{16}H_{11}ClN_4O_2$ (326.06). Calcd. (%): C, 58.82; H, 3.39; N, 17.15. Found (%): C, 59.11; H, 3.48; N, 17.37.

4.17 | Ethyl-3-(2-(3-Chloroquinoxalin-2-yl) hydrazineylidene)-butanoate 14g

To a suspension of the hydrazinyl derivative **3b** (0.19 g, 1 mmol) in absolute ethanol (15 mL), ethyl acetoacetate (0.13 g, 0.13 mL, 1 mmol) was added. The reaction mixture was then heated under reflux for 24 hours, concentrated, allowed to cool to form a precipitate that was crystallized from ethanol to produce 2-methylallyl propionate derivative **14g**. Brown powder, yield: (85%), m.p. > 300°C. IR (KBr, cm^{-1}): 3305 (NH); 3151, 3060 (CH-aromatic); 2992, 2933 (CH-aliphatic); 1725 (C═O); 1602 (C═N). ^{1}H NMR (DMSO-d_6) δ: 1.165 (t, 3H, $J = 8$ Hz, $CH_2\underline{CH_3}$); 1.190 (s, 3H, N═C—$\underline{CH_3}$); 2.271 (s, 2H, N═C—$\underline{CH_2}$); 4.061 (q, 2H, $J = 8$ Hz, $\underline{CH_2}CH_3$); 5.80 (s, NH, D2O exchangeable); 7.036 to 7.199 (m, 4H, quinoxaline $C_{5,6,7,8}$-H). ^{13}C NMR (DMSO-d_6) δ: 16.21 (N═C—$\underline{CH_3}$); 17.71 ($CH_2\underline{CH_3}$); 44.47 (N═C—$\underline{CH_2}$); 61.51 ($\underline{CH_2}$—$\underline{CH_3}$); 125.91, 126.53, 126.65, 129.19, 133.21, 143.34 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 143.52 (C—Cl); 159.99 (N═$\underline{C}$—$\underline{CH_3}$); 161.16 (quinoxaline C_3); 169.35 (COO). MS *m/z* (relative intensity %): 308 ($M^{+\cdot}$ + 2, 8.65); 306 ($M^{+\cdot}$, 24.04); 259 (79); 221 (68); 197 (80); 42 (100). Anal. form: $C_{14}H_{15}ClN_4O_2$ (306.09). Calcd. (%): C, 54.82; H, 4.93; N, 18.26. Found (%): C, 55.14; H, 5.19; N, 17.97.

4.18 | 3-(3-Amino-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)quinoxalin-2(1H)-one 15a and 5-Amino-2-(3-chloroquinoxalin-2-yl)-2,4-dihydro-3H-pyrazol-3-one 15b

To a suspension of the hydrazinyl derivative **3a** and **3b** (1 mmol) in methanol (15 mL), ethyl cyanoacetate (0.11 g, 0.11 mL, 1 mmol) and Na metal (0.02 g, 1 mmol) were added. The reaction mixture was then heated under reflux for 12 hours, concentrated, allowed to cool to form a precipitate that was recrystallized from ethanol to afford the oxopyrazole derivative **15a** and from DMF to afford **15b**; respectively.

4.19 | 3-(3-Amino-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)quinoxalin-2(1H)-one 15a

Brown crystals, yield: (89%), m.p.: 149°C to 150°C. IR (KBr, cm^{-1}): 3407 (OH-tautomer, —NH_2) 3174 (—NH); 1745, 1676 (two C═O); 1608 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, 2H, —NH_2, D_2O exchangeable); 2.66 (s, 2H, tautomeric CH_2); 7.02 to 7.14 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 12.07 (s, 1H, quinoxaline tautomeric NH, D_2O exchangeable). MS *m/z* (relative intensity %): 244 ($M^{+\cdot}$ +1, 24); 243 ($M^{+\cdot}$, 26); 159 (100); 111 (85); 80 (87). Anal. form: $C_{11}H_9N_5O_2$ (243.08). Calcd. (%): C, 54.32; H, 3.73; N, 28.79. Found (%): C, 54.61; H, 3.84; N, 29.04.

4.20 | 5-Amino-2-(3-chloroquinoxalin-2-yl)-2,4-dihydro-3H-pyrazol-3-one 15b

Black crystalline powder, yield: (69%), m.p. >300°C. IR (KBr, cm^{-1}): 3437 (NH_2); 3109, 3041 (CH-aromatic); 1643 (C═O); 1551 (C═N); 1483, 1406 (C═C); 764 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 2H, NH_2, D_2O exchangeable); 2.99 (s, 2H, tautomeric CH_2); 7.409 (t, 1H, $J = 8$ Hz, quinoxaline C_6-H); 7.42 (t, 1H, $J = 8$ Hz, quinoxaline C_7-H); 7.57 (d, 1H, $J = 8$ Hz, quinoxaline C_5-H); 7.83 (d, 1H, $J = 8$ Hz, quinoxaline C_8-H). MS *m/z* (relative intensity %): 263 ($M^{+\cdot}$ +2, 33); 261 ($M^{+\cdot}$, 69); 222 (100); 180 (87); 100 (84). Anal. form: $C_{11}H_8ClN_5O$ (261.04). Calcd. (%): C, 50.49; H, 3.08; N, 26.76. Found (%): C, 50.76; H, 3.17; N, 26.59.

4.21 | 3-(3,5-Dimethyl-1H-pyrazol-1-yl) quinoxalin-2(1H)-one 15c and2-Chloro-3-(3,5-dimethyl-1H-pyrazol-1-yl)quinoxaline 15d

An equimolar mixture of the hydrazinyl derivative **3a**,**b** (1 mmol) and acetylacetone (0.10 g, 0.10 mL, 1 mmol) was heated under reflux in ethanol for 22 to 25 hours. The reaction mixture was then concentrated and cooled at room temperature, and the precipitate formed was collected, dried, and crystallized from ethanol to produce the pyrazole derivatives **15c**,**d**.

4.22 | 3-(3,5-Dimethyl-1H-pyrazol-1-yl) quinoxalin-2(1H)-one 15c

Brick red powder yield: (75%), m.p. 79°C to 80°C. IR (KBr, cm^{-1}): 3568, 3450 (OH-tautomer); 3182, 3031 (CH-aromatic); 2969, 2914 (CH-aliphatic); 1677 (C═O). ^{1}H NMR (DMSO-d_6) δ: 2.19 (s, 3H, C_3-pyrazole $\underline{CH_3}$); 2.30 (s, 3H, C_5-pyrazole-$\underline{CH_3}$); 6.09 (s, 1H, pyrazole C_4-

H); 7.35 to 7.4 (m, 2H, quinoxaline $C_{7,8}$ H); 7.62 (t, 1H, J = 6 Hz, quinoxaline C_6-H); 7.78 (d, 1H, J = 6 Hz, quinoxaline C_5-H); 12.87 (s, 1H, quinoxaline-NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 12.01, 13.81 ($2CH_3$); 107.70 (pyrazole C_4); 123.47, 124.26, 128.79, 129.05. 130.78, 142.21 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 147.30 (pyrazole C_5); 149.46 (pyrazole C_3); 161.27 (quinoxaline-C_3); 164.11 (quinoxaline C═O). MS *m/z* (relative intensity %): 240 ($M^{+\cdot}$, 14); 211 (39); 198 (47); 121 (41.7); 44 (100). Anal. form: $C_{13}H_{12}N_4O$ (240.1). Calcd. (%): C, 64.99; H, 5.03; N, 23.32. Found (%): C, 64.78; H, 5.24; N, 23.50.

4.23 | 2-Chloro-3-(3,5-dimethyl-1H-pyrazol-1-yl)quinoxaline 15d

Brick red powder yield: (57%), m.p. 115°C to 116°C. IR (KBr, cm^{-1}): 3125, 3054 (CH-aromatic); 2930 (CH-aliphatic); 1568 (C═N); 1485, 1421 (C═C). ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, pyrazole C_3-CH_3); 2.30 (s, 3H, pyrazole C_5-CH_3); 6.05 (s, 1H, pyrazole C_4-H); 7.99 (t, 2H, J = 8 HZ, quinoxaline $C_{6,7}$ H); 8.17 (d, 1H, J = 8HZ, quinoxaline C_5-H); 8.18 (d, 1H, J = 8HZ, quinoxaline C_8-H). ^{13}C NMR (DMSO-d_6) δ: 11.89, 13.73 ($2CH_3$); 107.56 (pyrazole C_4); 122.71, 123.93, 127.85, 128.95, 129.93, 140.71 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a} of; respectively); 141.93 (pyrazole C_5-); 142.85 (pyrazole C_3); 149.59 (C—Cl); 155.19 (quinoxaline- C_3). MS *m/z* (relative intensity %): 260 ($M^{+\cdot}$ +2, 26); 258 ($M^{+\cdot}$, 7); 207 (67); 129 (75); 89 (100). Anal. form: $C_{13}H_{11}ClN_4$ (258.07). Calcd. (%): C, 55.29; H, 3.48; N, 21.49. Found (%): C, 55.58; H, 4.37; N, 21.89.

4.24 | 2-(3-Chloroquinoxalin-2-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one 15e

To a suspension of the hydrazinyl derivative **3b** (0.19 g, 1 mmol) in methanol (15 mL), ethyl acetoacetate (0.13 g, 0.13 mL, 1 mmol) and Na metal (0.02 g, 1 mmol) were added. The reaction mixture was then heated under reflux for 12 hours, concentrated, allowed to cool, triturated with ethanol to form a precipitate that was recrystallized from DMF to afford the oxopyrazole derivative **15e**. White crystalline powder, yield: (71%), m.p. >300°C. IR (KBr, cm^{-1}): 3063 (CH-aromatic); 2990, 2912, 2855 (CH-aliphatic); 1708 (C═O); 1619 (C═N). ^{1}H NMR (DMSO-d_6) δ: 1.243 (s, 3H, CH_3); 1.579 (s, 2H, CH_2); 7.115 to 7.298 (m, 4H, quinoxaline $C_{5,6,7,8}$-H). ^{13}C NMR (DMSO-d_6) δ: 24.04 (CH_3); 47.17 (CH_2); 125.99, 126.57, 126.60, 129.09, 133.13, 143.34 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 143.92 (C—Cl); 159.78 (C—CH_3); 161.01 (quinoxaline C_3); 170.23 (C═O). MS *m/z* (relative intensity %): 262 ($M^{+\cdot}$ +2, 14); 260 ($M^{+\cdot}$, 20); 195 (24); 167 (33); 54 (100). Anal. form: $C_{12}H_9ClN_4O$ (260.68). Calcd. (%):C, 55.29; H, 3.48; N, 21.49. Found (%): C, 55.53; H, 3.62; N, 21.75.

4.25 | 2-Chloro-N′-(3-substituted-quinoxalin-2-yl)acetohydrazide 16a,b

To a well-stirred solution of the hydrazinyl derivative **3a, b** (1 mmol) in dimethylformamide (5 mL), chloroacetyl chloride (0.11 g, 0.08 mL, 1 mmol) was added dropwise while stirring. The reaction mixture was then left to be stirred overnight at room temperature, the obtained precipitate was filtered, washed with ethanol, dried, and crystallized from DMF to yield the acetohydrazide derivatives **16a,b**.

4.26 | 2-Chloro-N′-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetohydrazide 16a

Yellow powder, yield: (61%), m.p. 249°C to 250°C. IR (KBr, cm^{-1}): 3437 (OH tautomer); 3039 (CH-aromatic); 2904 (CH-aliphatic); 1680 (C═O); 1616 (C═C); 758 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.73 (s, 1H, NH—NH-CO, D_2O exchangeable); 5.57 (s, 2H, CH_2); 7.36 to 7.53 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 12.19 (s, 2H, OH and NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 37.11 (CH_2); 123.16, 123.83, 127.90, 128.62, 129.88, 145.71 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}, respectively); 160.02 (quinoxaline C_3); 162.78 (quinoxaline C═O); 165.75 (amidic C═O). MS *m/z* (relative intensity %): 254 ($M^{+\cdot}$ +2, 8); 252 ($M^{+\cdot}$, 10.6); 157 (20); 105 (46.9); 44 (100). Anal. form: $C_{10}H_9ClN_4O$ (252.04). Calcd. (%): C, 47.54; H, 3.59; N, 22.18. Found (%): C, 47.78; H, 3.71; N, 22.45.

4.27 | 2-Chloro-N′-(3-chloroquinoxalin-2-yl)acetohydrazide; 16b

Yellow crystals, yield: (76%), m.p. >300°C. IR (KBr, cm^{-1}): 3416 (OH-tautomer, NH); 3050 (CH-aromatic); 2995, 2834 (CH-aliphatic); 1628 (C═O); 1484 (C═C); 756 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 3.34 (s, 1H, NH—NH-CO, D_2O exchangeable); 5.66 (s, 2H, CH_2); 7.75 to 7.84 (m, 2H, quinoxaline $C_{6,7}$- H); 8.33 (d, 1H, J = 8 Hz, quinoxaline C_8-H); 8.37 (d, 1H, J = 8 Hz, quinoxaline C_5 -H); 10.05 (s, 1H, amidic NH, D_2O exchangeable). MS *m/z* (relative intensity %): 274 ($M^{+\cdot}$ +4, 2); 272 ($M^{+\cdot}$ +2, 24); 270 ($M^{+\cdot}$, 66); 101 (53); 89 (88); 75 (100). Anal. form: $C_{10}H_8Cl_2N_4O$ (270.01). Calcd. (%): C, 44.30; H, 2.97; N, 20.67. Found (%): C, 44.53; H, 3.14; N, 20.89.

4.28 | 3-(2-(2-Oxo-2-phenylethyl) hydrazineyl)quinoxalin-2(1H)-one 16c and 2-(2-(3-chloroquinoxalin-2-yl)hydrazineyl)-1-phenylethan-1-one 16d

To a well-stirred solution of the hydrazinyl derivatives **3a** and **3b** (1 mmol) in dioxane (15 mL), phenacyl bromide (0.2 g, 1 mmol) was added while stirring. The reaction mixture was then left to be stirred for 2 hours at room temperature, then refluxed for 12 hours, concentrated, allowed to cool to form a precipitate that was washed with ether and crystallized from dioxan to give compound **16c** and from ethanol to afford compound **16d**, respectively.

4.29 | 3-(2-(2-Oxo-2-phenylethyl) hydrazineyl)quinoxalin-2(1H)-one 16c

Yellow crystals, yield: (56%), m.p. 189°C to 190°C. IR (KBr, cm^{-1}): 3405 (NH); 3100 (CH-aromatic); 2984, 2913 (CH-aliphatic); 1693 (C═O). ^{1}H NMR (DMSO-d_6) δ: 2.50 (s, 2H, —NH—NH—, D_2O exchangeable); 4.52 (s, 2H, CH_2); 7.47 to 7.52 (m, 4H, quinoxaline $C_{5,6,7,8}$-H);7.58 to 7.68 (m, 3H, phenyl $C_{3,4,5}$ -H);8.08 (d, 1H, J = 8 Hz, phenyl C_1-H); 8.14 (d, 1H, J = 8 Hz, phenyl C_6-H); 13.27 (s, 1H, amidic NH, D_2O exchangeable). MS *m/z* (relative intensity %): 294 ($M^{+\cdot}$, 19); 276 (100); 205 (24); 164 (78); 105 (75). Anal. form: $C_{16}H_{14}N_4O_2$ (294.11). Calcd. (%): C, 65.30; H, 4.79; N, 19.04. Found (%): C, 65.49; H, 4.85; N, 19.21.

4.30 | 2-(2-(3-chloroquinoxalin-2-yl) hydrazineyl)-1-phenylethan-1-one 16d

Yellow crystals, yield: (63%), m.p. 194°C to 195°C. IR (KBr, cm^{-1}): 3413 (NH); 3144 (CH-aromatic); 1679 (C═O); 1607, 1545 (C═N); 1482, 1402 (C═C); 753 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 1H, —NH—CH_2, D_2O exchangeable); 3.49 (s, 1H, NH—NH—CH_2, D_2O exchangeable); 4.46 (s, 2H, CH_2); 7.15 to 7.51 (m, 9H, phenyl $C_{2,3,4,5,6}$-H, quinoxaline $C_{5,6,7,8}$-H). MS *m/z* (relative intensity %): 314 ($M^{+\cdot}$ +2, 2); 312 ($M^{+\cdot}$, 7); 272 (12); 102 (26); 76 (100). Anal. form: $C_{16}H_{13}ClN_4O$ (312.08). Calcd. (%): C, 61.44; H, 4.19; N, 17.91. Found (%): C, 61.72; H, 4.28; N, 18.04.

4.31 | 2-(2-(3-Substituted-2-yl)hydrazine-1-carbonyl)benzoic acid 16e and 16f

An equimolar mixture of the hydrazinyl derivative **3a,b** (1 mmol) and phthalic anhydride (0.15 g, 1 mmol) was heated under reflux in glacial acetic acid (20 mL) for 14 hours. The reaction mixture was then concentrated and cooled to room temperature, and the precipitate formed was collected, dried, and crystallized from ethanol to give compound **16c** and from DMF to give compound **16f**, respectively.

4.32 | 2-(2-(3-Oxo-3,4-dihydroquinoxalin-2-yl)hydrazine-1-carbonyl)benzoic acid 16e

Yellow powder, yield: (89%), m.p. >300°C. IR (KBr, cm^{-1}): 3385 (br, OH and NH); 1733, 1677 (2 C═O). ^{1}H NMR (DMSO-d_6) δ: 2.5 (1H, NH, D_2O exchangeable); 7.20 to 7.46 (m, 4H, quinoxaline-$C_{5,6,7,8}$-H); 7.69 to 8.05 (m, 4H, benzoic acid $C_{3,4,5,6}$-H); 11.99 (s, 1H, OH, D_2O exchangeable); 12.59 (s, 2H, OH and NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 123.60, 124.02, 124.32, 126.04, 126.18, 127.95, 129.51, 129.60, 129.89, 131.81, 135.87, 144.71 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a} and phenyl ring); 161.99 (quinoxaline C_3); 162.99 (quinoxaline C═O); 165.96 (amidic C═O); 169.19 (COOH). MS *m/z* (relative intensity %): 324 ($M^{+\cdot}$, 12.7); 289 (32); 239 (58.7); 197 (28.6); 104 (100). Anal. form: $C_{16}H_{10}N_4O_3$ (324.29). Calcd. (%): C, 59.26; H, 3.73; N, 17.28. Found (%): C, 59.60; H, 3.85; N, 17.45.

4.33 | 2-(2-(3-Chloroquinoxalin-2-yl) hydrazine-1-carbonyl)-benzoic acid 16f

Brown powder, yield: (79%), m.p. >300°C. IR (KBr, cm^{-1}): 3433 (br. OH, NH); 3130 (CH-aromatic); 1715, 1635 (2C═O); 1488, 1407 (C═C). ^{1}H NMR (DMSO-d_6) δ: 3.34 (s, 1H, NH, D_2O exchangeable); 7.47 (d, 1H, J = 8 HZ, phenyl C_6-H); 7.57 (t, 1H, J = 8 Hz, phenyl C_5-H); 7.68 (t, 1H, J = 8 Hz, quinoxaline C_6-H); 7.87 (t, 2H, J = 8 Hz, phenyl C_4-H and quinoxaline C_7-H); 8.05 (d, 1H, J = 8 Hz, quinoxaline C_5-H); 8.20 (d, 1H, J = 8 Hz, quinoxaline C_8-H); 8.32 (d, 1H, J = 8 Hz, phenyl C_3-H); 11.00 (s, 1H, amidic-NH, D_2O exchangeable); 11.59 (s, 1H, -COOH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 124.41, 125.01, 125.59, 125.86, 127.70, 127.91 (quinoxaline- C_5, C_8, C_6, C_7, C_{8a}, C_{4a}; respectively); 129.88, 131.02, 133.04, 135.94 (phenyl -$C_{1,\ 2,\ 3,\ 4,\ 5,\ 6}$); 138.00 (C-CONH); 144.72 (C—COOH); 148.89 (C—Cl); 162.31 (quinoxaline C_3); 166.03 (C═O); 169.43 (COOH). MS *m/z* (relative intensity %): 344 ($M^{+\cdot}$ + 2, 100); 342 ($M^{+\cdot}$, 5); 299 (70); 102 (24). Anal. form: $C_{16}H_{11}ClN_4O_3$ (342.05). Calcd. (%): C, 56.07; H, 3.23; N, 16.35. Found (%) C, 56.38; H, 3.49; N, 16.46.

4.34 | 2-(Substituted-2-yl)hydrazine-1-carboximidamide 16 g,h

A mixture of the hydrazinyl derivatives **3a**,**b** (1 mmol) and cyanamide (0.08 g, 2 mmol) was heated under reflux in absolute ethanol (15 mL) acidified with conc. HCl (5 drops) for 12 h. The reaction mixture was then concentrated, allowed to attain room temperature, and the precipitate formed was collected, dried, and crystallized from DMF to produce the guanidine derivative **16g** and from ethanol to afford the compound **16h**, respectively.

4.35 | 2-(3-Oxo-3,4-dihydroquinoxalin-2-yl) hydrazine-1-carboximidamide 16g

Brick red powder yield: (37%), m.p. >300°C. Found (%): C, 49.35; H, 4.80; N, 38.79. IR (KBr, cm^{-1}): 3429 (OH-tautomer); 3318 to 3370 (—NH_2, 3-NH); 3115, 3042 (CH-aromatic); 1666 (C═O); 1615 (C═N). ^1H NMR (DMSO-d_6) δ: 2.50 (s, 4H, —NH_2 and NH—NH, D_2O exchangeable); 6.22 (s, 1H, —C═NH, D_2O exchangeable); 7.02 to 7.17 (m, 4H, quinoxaline $C_{5,6,7,8}$-H); 11.96 (s, 1H, quinoxaline NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 123.43, 124.66, 125.16, 129.29, 130.88, 148.95 (quinoxaline C_5, C_8, C_6, C_7, C_{8a}, C_{4a}, respectively); 161.45 (quinoxaline C_3); 163.05 (C (NH) NH_2); 163.93 (quinoxaline C═O). MS *m/z* (relative intensity %): 219 ($M^{+\cdot}$ +1, 13); 218 ($M^{+\cdot}$, 23); 134 (71); 106 (100); 76 (99). Anal. form: $C_9H_{10}N_6O$ (218.09). Calcd. (%): C, 49.54; H, 4.62; N, 38.51.

4.36 | (3-Chloroquinoxalin-2-yl)hydrazine-1-carboximidamide 16h

Red powder yield: (51%), m.p. 159°C to 160°C. Anal. form: $C_9H_9ClN_6$ (236.06). Calcd. (%): C, 45.68; H, 3.83; N, 35.51. Found (%): C, 46.01; H, 3.97; N, 35.80. IR (KBr, cm^{-1}): 3369 (NH, NH_2); 3152 (CH-aromatic); 1625, 1565 (C═N); 1474 (C═C); 749 (C—Cl). ^1H NMR (DMSO-d_6) δ: 1.04 (s, 1H, NH—C═NH, D_2O exchangeable); 1.55 (s, 2H, NH_2, D_2O exchangeable); 4.72 (s, 1H, NH—NH-C (NH), D_2O exchangeable); 5.30 (s, 1H, —C═NH, D_2O exchangeable); 7.02 to 7.69 (m, 4H, quinoxaline $C_{5,6,7,8}$-H). MS *m/z* (relative intensity %): 238 ($M^{+\cdot}$ +2, 1.5); 236 ($M^{+\cdot}$, 40); 186 (38); 171 (100); 62 (60).

ORCID

Maha A. Ebrahim https://orcid.org/0000-0001-5877-0671

REFERENCES AND NOTES

[1] U. Natarajan, I. Kaliappan, N. K. Singh, *Der Pharma Chemica* **2010**, *2*, 159.

[2] N. Umarani, K. Ilango, *Int J Pharm Sci Rev Res* **2010**, *2*, 24.

[3] I. Iskdag, Y. Ozkay, Z. Incesu, *Turk J Pharm Sci* **2011**, *8*, 179.

[4] M. A. Amin, M. M. Youssef, *Der Pharma Chemica* **2012**, *4*, 1323.

[5] V. Kotra, K. Pradeep, R. Vasanthi, *Der Pharma Chemica* **2013**, *5*, 301.

[6] E. Elkaeed, A. Ghiaty, A. El-Morsy, K. El-Gamal, H. Sakr, *Chem Sci Rev Lett* **2014**, *3*, 1375.

[7] F. A. S. Alasmari, N. A. Aljaber, M. M. Korrah, *IJARCS* **2015**, *2*, 14.

[8] R. Padma, S. Guhanathan, *Pelagia Research Library-Der Chemica Sinica* **2016**, *7*, 63.

[9] L. Alexander, M. Eyal, *AnnuRevBiochem* **2006**, *75*, 93. https://doi.org/10.1146/annurev.biochem.75.103004.142657

[10] P. K. Dubey, V. S. H. Krishnan, K. S. Chowdary, *Indian J Chem* **2000**, *39B*, 329.

[11] D. R. Romer, *J Heterocyclic Chem* **2009**, *46*, 317.

[12] C. Chen, J. Chen, H. Gu, N. Bao, H. Dai, *Molecules* **2017**, *22*, 1126, 1.

[13] A. A. Fadda, S. Bondock, R. Rabie, H. A. Etman, *Turk J Chem* **2008**, *32*, 259.

[14] A. A. Farag, *Der Pharma Chemica* **2015**, *7*, 130.

[15] E. S. Darwish, K. A. Atia, A. M. Farag, *Heterocycles* **2014**, *89*, 1393.

[16] S. A. Galal, A. S. Abdelsamie, H. Tokuda, N. Suzuki, A. Lida, M. M. El-Hefnawi, R. A. Ramadan, M. H. E. Atta, H. I. El Diwani, *Eur J Med Chem* **2011**, *46*, 327.

[17] S. Parraa, F. Laurentb, G. Subraa, C. D. Masquefaa, V. Benezecha, J. R. Fabreguettesa, J. P. Vidalc, T. Pocockd, K. Elliottd, R. Smalld, R. Escalea, A. Michelb, J. P. Chapata, P. A. Bonneta, *Eur J Med Chem* **2001**, *36*, 255.

[18] H. Saikachi, S. Tagami, *Chem Pharm Bull* **1961**, *9*, 941.

[19] X. K. Liu, L. X. Ma, Z. Y. Wei, X. Cui, S. Zhan, X. M. Yin, H. R. Piao, *Molecules* **2017**, *22*, 273, 1.

[20] A. H. R. Betrow, K. M. Hamza, R. M. Tarhoun, *Univ Bulletin* **2013**, *15*.

[21] F. A. Yassin, *J Microbiol Antimicrob* **2010**, *2*, 93.

[22] Z. M. Nofal, H. H. Fahmy, E. S. Zarea, W. El-Eraky, *Acta Pol Pharm* **2011**, *68*, 507.

[23] L. J. Hu, Z. J. Zhan, M. Lei, L. H. Hu, *Arkivoc* **2013**, 189.

[24] M. H. Ashokbhai, P. J. Merambhai, P. K. Narbherambhai, K. K. Kapuriya, P. K. Patel, *Arch Appl Sci* **2014**, *6*, 74.

[25] T. Singh, S. Sharma, V. K. Srivastava, *Indian J Chem B* **2006**, *45*, 1557.

[26] S. M. Mosaad, K. I. Mohammed, M. A. Ahmed, S. G. Abdel-Hamide, *J Biol Sci* **2004**, *4*, 504.

[27] W. A. Bayoumi, A. M. Barghash, M. M. Gineinah, M. A. Massoud, A. M. Abdelal, *Der Pharma Chemica* **2014**, *6*, 89.

[28] F. Al-Omran, A. Abou El-Khir, *J Org Chem* **2016**, *6*, 31.

[29] K. M. Amin, F. M. Awadallah, A. A. M. Eissa, S. M. Abou-Seri, G. S. Hassan, *Bioorg Med Chem* **2011**, *19*, 6087.

[30] C. Valgas, S. M. Souza, E. F. A. Smânia, A. Smânia, *Braz J Microbiol* **2007**, *38*, 369.

[31] A. Sen, A. Batra, *Int J Curr Pharm Res* **2012**, *4*, 67.

[32] M. A. Hussain, A. Shah, I. Jantan, M. N. Tahir, M. R. Shah, R. Ahmed, S. N. A. Bukhari, *J Nanobiotechnol* **2014**, *12*, 1.

[33] A. Sharma, K. Sharma, *Adv Biol Res* **2011**, *5*, 241.

[34] R. T. Al- Naama, *J Bacteriol Res* **2009**, *1*, 064.

[35] M. M. Tunney, G. Ramage, R. T. Field, T. F. Moriarty, D. G. Story, *Antimicrob Agents Chemother* **2004**, *48*, 1879.

[36] H. A. Mohammed, M. M. Abdel-Aziz, M. M. Hegazy, *Medicina* **2019**, *55*, 301, 1.

[37] S. Kumar, S. Bajaj, R. B. Bodla, *Indian J Pharm* **2016**, *48*, 481.

[38] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv Drug Deliv Rev* **2001**, *46*, 3.

[39] S. Ekins, S. Andreyev, A. Ryabov, E. Kirillov, E. A. Rakhmatulin, S. Sorokina, *Drug Metab Dispos* **2005**, *34*, 495.

How to cite this article: Bayoumi AH, Ghiaty AH, Abd El-Gilil SM, Husseiny EM, Ebrahim MA. Exploration of quinoxaline derivatives as antimicrobial and anticancer agents. *J Heterocyclic Chem*. 2019;1–21. https://doi.org/10.1002/jhet.3716