Medicinal Chemistry Research (2018) 27:2287–2296
https://doi.org/10.1007/s00044-018-2235-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and biological evaluation of quinoxaline *N*-propionic and *O*-propionic hydrazide derivatives as antibacterial and antifungal agents

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Received: 4 June 2018 / Accepted: 7 August 2018 / Published online: 17 August 2018

Abstract

Novel series of quinaxoline derivatives incorporating *N*-propionic and *O*-propionic hydrazide moieties were synthesized. Alkylation of 3-methylquinoxalin-2(1*H*)-one with ethyl 2-bromopropanoate afforded a mixture of *O*-alkylated and *N*-alkylated 3-methylquinoxaline. Hydrazide derivatives were afforded by reaction of *O*-alkylated and *N*-alkylated 3-methylquinoxaline with hydrazine hydrate. Condensation of hydrazide derivatives with different aromatic aldehydes and formylpyrazoles afforded the corresponding hydrazone derivatives. The synthesized quinaxoline derivatives were evaluated for their expected antimicrobial activity; where, the majority of these compounds showed potent antibacterial and antifungal activities against the tested strains of bacteria and fungi. Hydrazone derivative which contain 3-*p*-tolyl-pyrazolyl moiety showed fourfold potency of amphotericin B in inhibiting the growth of *Aspergillus fumigatus*, twofold potency of gentamycin in inhibiting the growth of *Neisseria gonorrhoeae*, equipotent potency of ampicillin in inhibiting the growth of *Streptococcus pyogenes*, equipotent potency of gentamycin in inhibiting the growth of *Proteus vulgaris* and *Shigella flexneri*, equipotent potency of amphotericin B in inhibiting the growth of *Aspergillus clavatus*, *Geotrichum candidum* and *Penicillium marneffei*. Thus, these studies suggested that quinaxoline derivatives bearing a pyrazole moiety are interesting scaffolds for the development of novel antibacterial and antifungal agents.

Keywords Quinaxolines · Propionic acid derivatives · Hydrazone derivatives · Antibacterial and antifungal activities

Introduction

Antimicrobial agents are the most significant weapons in fighting bacterial and fungal infections. The development of the bacterial and fungal resistant to many current antimicrobial agents do increase our interest for the discovery of new antibacterial and antifungal agents. Moreover, infections caused by multi-drug-resistant bacteria and fungi are difficult to diagnose and treat (El Shehry et al. 2018; Abbas et al. 2013, 2018; El-Sharief et al. 2016; Moussa et al. 2016; Fouad et al. 2018; Hessein et al. 2016). So, development and discovery of new antimicrobial drugs are urgently needed to overcome the growing of drug-resistant microbes.

Quinoxaline ring systems are attractive candidates in medicinal chemistry. They constitute the building blocks for many natural and synthetic pharmacologically active compounds. Quinoxaline ring is a part of the naturally occurring antibiotics, such as echinomycin, triostin A. echinoserinethat, and quinaldopeptin. Also, quinoxaline derivatives

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00044-018-2235-4) contains supplementary material, which is available to authorized users.

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possess antiviral, antihistaminic, anticancer, antiinflammatory, and antidiabetic activities (Al-Marhabi et al. 2015; Ammar et al. 2016; Amin et al. 2006; Ismail et al. 2010; Abbas et al. 2015).

Hydrazide–hydrazone derivatives are among the most important designed moiety for discovering an effective compound against multidrug-resistant microbial infection. Hydrazides have been demonstrated to possess antimycobacterial (Angelova et al. 2017), antifungal (Kauthale et al. 2017), anti-HIV-1 (Yang et al. 2016), and antihepatitis C virus (Senkardes et al. 2016) activities. They also have leishmanicidal and trypanocidal activities (Coa et al. 2015; Palace-Berl et al. 2018) as well as anticancer (Islam et al. 2017) and insecticidal (Yang et al. 2016) activities.

In view of the above-mentioned results and as a continuation of our research on quinoxaline derivatives in an attempt to identify new lead compounds that might be of value for future development as new class of antimicrobial agents, we report herein the synthesis and antimicrobial evaluation of a new series of quinoxaline derivatives linked through hydrazide–hydrazone function (Fig. 1) in order to achieve further knowledge of the structure–activity relationship.

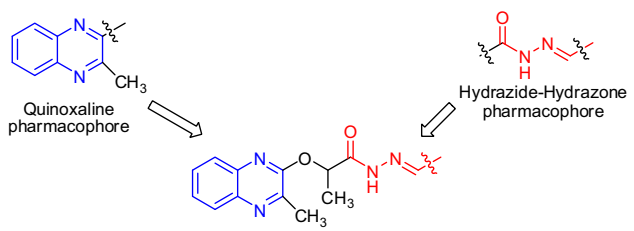
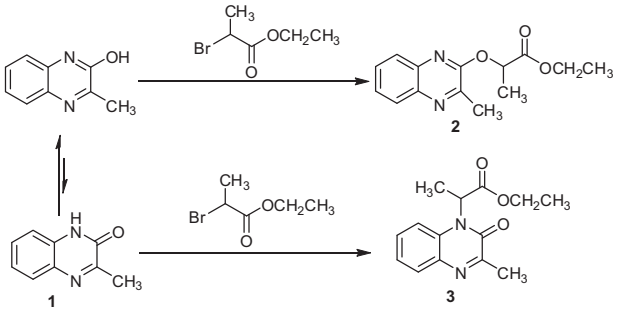


Fig. 1 Design of quinoxaline hybrids hydrazide–hydrazones antimicrobial agents

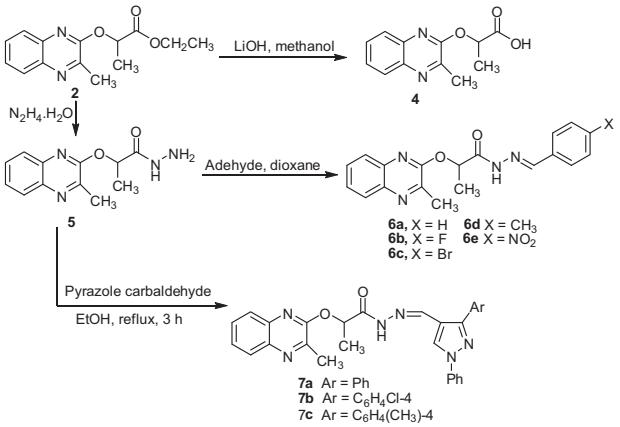
Results and discussion

Chemistry

The starting material, 3-methylquinoxalin-2(1*H*)-one (**1**) was proven to be a good synthon for different highly biologically active compounds. 3-Methylquinoxalin-2(1*H*)-one (**1**) having an amide function can exhibit one-ol tautomerism. Compound **1** was alkylated with ethyl 2-bromopropanoate in acetone containing potassium carbonate as catalyst. The reaction was monitored by TLC which indicated that there is a mixture of two products, the two products were confirmed as the *O*-alkylated **2** and *N*-alkylated **3** of 3-methylquinoxaline (Scheme 1). The structures of the reaction products were confirmed based on spectral and analytical data. IR measurement of **2** showed one



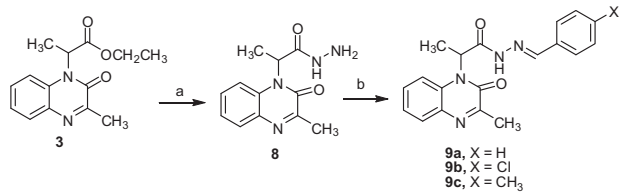
Scheme 1 Synthesis of *O*-alkylated **2** and *N*-alkylated **3** of 3-methylquinoxaline



Scheme 2 Syntheses of propanoic acid derivative **4**, hydrazide derivative **5** and hydrazone derivatives **6a–e**, **7a–c**

diagnostic absorbance band at 1752 cm^{-1} for one C=O functional group. While IR measurement of **3** showed two diagnostic absorbance bands at 1744 and 1659 cm^{-1} for two C=O functional groups. ^{1}H NMR of **2** exhibited five diagnostic signals at: 1.29, 1.75, 2.75, 4.17–4.33, and 5.50 ppm characterized for CH_3–ester, CH_3–CH, CH_3–quinoxaline, CH_2–ester, and CH–O protons, respectively. ^{1}H NMR of **3** exhibited signal at 5.76 ppm characterized for CH–N proton.^{13}C NMR of **2**, this signal resonated in the deshielded region of 70.6 ppm are assigned to CH–O carbon while in ^{13}C NMR of **3** exhibited signal at 51.3 characterized for CH–N carbon.

β-Propanoic acid derivative **4** was obtained in good yield and high purity by hydrolysis of ester group in compound **2** using LiOH in ethanol. All spectral data were in accordance with its structure (Scheme 2). Hydrazide derivative **5** was afforded by reaction of ethyl propanoate derivative **2** with hydrazine hydrate. IR spectrum of hydrazide derivative **5** showed strong peaks at 3334 and 3282 cm^{-1} indicating the presence of $–NHNH_2$ group, also NMR spectra were in accordance with its structure. Condensation of hydrazide derivative **5** with different aromatic aldehydes afforded the corresponding hydrazone derivatives **6a–e**. Condensation of the hydrazide **5** with formylpyrazole derivatives in ethanol



Scheme 3 Syntheses of hydrazide derivative **8** and hydrazone derivatives **9a–c**; Reagents and conditions: (**a**) LiOH, methanol; (**b**) 4-nitrobenzaldehyde, acetic acid; (**c**) hydrazine hydrate, ethanol; (**d**) aldehyde, dioxane; and (**e**) pyrazolecarbaldehyde, EtOH, reflux, 3 h

under reflux afforded the respective hydrazone derivatives **7a–c**. The structures of products were deduced from elemental analysis and spectroscopic data. Most of hydrazone derivatives show two sets of signals in NMR experiments.

Moreover, treatment of ethyl propanoate derivative **3** with hydrazine hydrate afforded hydrazide derivative **8**. Its IR spectrum showed strong peaks at 3456, 3354, and 3264 cm^{-1} indicating the presence of $–NHNH_2$ group, also NMR spectra were in accordance with its structure. Condensation of hydrazide derivative **8** with different aromatic aldehydes afforded the corresponding hydrazone derivatives **9a–c**. Most hydrazone derivatives show two sets of signals in NMR experiments (Scheme 3).

Antibacterial and antifungal activities

The present investigation started with design and syntheses of new series of quinaxoline derivatives. Thus, quinaxoline derivative 2 which bearing *β*-propionate at C-(3) was synthesized; this in order to study the effect of *β*-propionate at C-(3) of the quinaxoline scaffold on the antimicrobial activity. So, changing the substituent on C-(3) from *β*-propionate to *β*-propionic acid (**3 → 4**) was carried out to show the difference between *β*-propionate and *β*-propionic acid on the effect of the antimicrobial activities. Also, changing the substituent on C-(3) of quinaxoline moiety from *β*-propionate to hydrazide (**2 → 5**) was carried out for the same reason. A series of hydrazide derivatives **6a–e** which contain various substituent at phenyl ring was synthesized. The effect of each substituent on the antimicrobial activity was studied. So, changing the substituent on from hydrogen to fluorine to bromine to methyl to nitro group (**6a → 6b → 6c → 6d → 6e**) was carried out to show the difference between H, F, Br, CH_3, and NO_2 on the effect of the biological activities. Another series of hydrazide derivatives **7a–c** which contain substituent pyrazole was synthesized. Changing the substituent on pyrazole from phenyl to 4-chlorophenyl to 4-methylphenyl (**7a → 7b → 7c**) was carried out for the same reason.

By the same way, quinaxoline derivative **3**, which is bearing *β*-propionate at N-(1) was synthesized; this is in order to study the effect of *β*-propionate at N-(1) of the quinaxoline scaffold on the antimicrobial activity. Changing the substituent on N-(1) from *β*-propionate to hydrazide (**2 → 8**) was carried out for the same reason. A series of hydrazide derivatives **9a–c** which contain various substituents at phenyl ring was synthesized in order to study the difference between H, Cl, and CH_3 on the effect of the biological activities.

The newly synthesized target compounds were evaluated in vitro for their expected antibacterial and antifungal activities. Sixteen test organisms representing three different microbial groups were used: Group 1: (Gram-positive bacteria) *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus pyogenes* (RCMB 010015), *Bacillis subtilis* (RCMB 010063), and *Enterococcus faecalis* (RCMB 010068); Group 2: (Gram-negative bacteria) *Neisseria gonorrhoeae* (RCMB 010076), *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumonia* (RCMB 010093), *Shigella flexneri* (RCMB 0100542), and *Pseudomonas aeruginosa* (RCMB 010043); Group 3: (Fungi) *Aspergillus fumigates* (RCMB 02564), *Aspergillus clavatus* (RCMB02593), *Candida albicans* (RCMB 05035), *Geotrichum candidum* (RCMB 05096), *Penicillium marneffei* (RCMB 01267), and *Syncephalastrumr acemosum* (RCMB 05922). Agar-diffusion method (Cooper 1972) was used for the determination of the preliminary screening of antibacterial activity. The newly synthesized target compounds were evaluated against clinical isolates of standard strains of fungi by the broth dilution method according to NCCLs (Abbas et al. 2013). Ampicillin, gentamycin, and amphotericin B were used as reference drugs for Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. The experiment was carried out in triplicate and the zones of inhibition were recorded for each tested compounds as the average diameter of inhibition zones of bacterial or fungal growth around the discs in mm. The inhibition zone diameters, attributed to the tested original concentration (5 mg/mL) as a preliminary test, are shown in Tables 1–3 and represented graphically in Fig. 2.

The key precursor, quinaxoline derivative **2**, which is bearing *β*-propionate at C-(3) showed weak antimicrobial activity as revealed from its inhibition zones (0–15.2 mm).

Changing the substituent on C-(3) of quinaxoline moiety from *β*-propionate to hydrazide (**2 → 5**) showed little improvement in antibacterial and antifungal activities. The results displayed this hydrazide derivative **5** showed moderate activity against most of all the screened organisms.

Treatment of the hydrazide derivative with aromatic aldehydes improves the antibacterial and antifungal activities, where the results revealed that the antimicrobial activity of some hydrazone derivative were nearly equipotent to the reference drug against all screened organisms. Structure **6** has at position-3 side chain ending with aryl

Table 1 Antibacterial activity of the synthesized compounds and standard reference against the pathological Gram-positive bacteria expressed as inhibition diameter zones in millimeters (mm)

Compd. no.	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*B. subtilis*	*E. faecalis*
2	14.2 ± 0.15	13.7 ± 0.43	NA	14.21 ± 0.3	NA
3	15.1 ± 0.76	14.5 ± 0.56	NA	13.1 ± 0.33	10.0 ± 0.23
5	17.2 ± 0.43	14.2 ± 0.24	NA	18.4 ± 0.15	11.1 ± 0.34
6a	24.5 ± 0.36	22.5 ± 0.43	23.7 ± 0.87	26.3 ± 0.67	20.3 ± 0.43
6b	**29.5 ± 0.78**	**26.8 ± 0.45**	**27.5 ± 0.43**	**35.7 ± 0.76**	**24.2 ± 0.43**
6c	25.6 ± 0.56	22.4 ± 0.50	21.4 ± 0.22	28.6 ± 0.57	20.5 ± 0.54
6d	24 ± 0.51	20.9 ± 0.63	18.9 ± 0.34	24.8 ± 0.27	18.1 ± 0.30
6e	**27.8 ± 0.25**	23.2 ± 0.57	23.2 ± 0.32	27.9 ± 0.44	20.1 ± 0.30
7a	**29.9 ± 0.45**	**27.6 ± 0.45**	**28.5 ± 0.43**	**34.8 ± 0.38**	**23.8 ± 0.38**
7b	**29.2 ± 0.32**	**26.1 ± 0.38**	**28.3 ± 0.43**	**36.3 ± 0.43**	**23.6 ± 0.45**
7c	**30.0 ± 0.54**	**28.2 ± 0.54**	**28.5 ± 0.32**	**32.9 ± 0.34**	**26.4 ± 0.37**
8	15.3 ± 0.43	14.7 ± 0.43	NA	17.2 ± 0.22	13.2 ± 0.35
9a	17.7 ± 0.33	14.4 ± 0.21	NA	16.5 ± 0.41	10.5 ± 0.54
9b	26.7 ± 0.65	22.0 ± 0.29	21.7 ± 0.34	28.8 ± 0.39	21.3 ± 0.24
9c	24.3 ± 0.52	23.3 ± 0.53	24.2 ± 0.38	28.3 ± 0.54	20.4 ± 0.46
Ampicillin	28.9 ± 0.14	25.4 ± 0.18	26.4 ± 0.34	34.6 ± 0.35	22.6 ± 0.31

The meaning of the bold values is these results are promising results

Table 2 Antibacterial activity of the synthesized compounds and standard reference against the pathological Gram-negative bacteria expressed as inhibition diameter zones in millimeters (mm)

Compd. no.	*N. gonorrhoeae*	*P. vulgaris*	*K. pneumonia*	*S. flexneri*	*P. aeruginosa*
2	NA	12.3 ± 0.56	15.2 ± 0.62	NA	NA
3	NA	12.3 ± 0.24	13.5 ± 0.54	NA	NA
5	NA	12.5 ± 0.52	13.9 ± 0.46	11.9 ± 0.34	NA
6a	**19.7 ± 0.37**	22.6 ± 0.23	21.0 ± 0.62	21.4 ± 0.62	16.3 ± 0.51
6b	**20.4 ± 0.43**	**24.5 ± 0.23**	**27.7 ± 0.35**	**25.2 ± 0.65**	**18.2 ± 0.45**
6c	17.6 ± 0.34	20.2 ± 0.32	23.4 ± 0.54	22.4 ± 0.33	15.0 ± 0.23
6d	**18.2 ± 0.52**	21.2 ± 0.74	22.8 ± 0.25	21.6 ± 0.56	16.8 ± 0.64
6e	**18.7 ± 0.34**	21.2 ± 0.75	23.8 ± 0.37	22.9 ± 0.35	16.6 ± 0.42
7a	**21.0 ± 0.21**	**23.8 ± 0.24**	28.2 ± 0.34	**25.5 ± 0.39**	**19.4 ± 0.46**
7b	**22.7 ± 0.56**	**25.1 ± 0.54**	**27.2 ± 0.19**	**26.2 ± 0.33**	**18.8 ± 0.32**
7c	**20.3 ± 0.16**	**25.9 ± 0.17**	**29.2 ± 0.27**	**26.2 ± 0.65**	**17.8 ± 0.45**
8	NA	NA	NA	NA	NA
9a	12.2 ± 0.41	14.8 ± 0.43	15.5 ± 0.76	14.7 ± 0.43	NA
9b	**18.7 ± 0.59**	**22.5 ± 0.36**	24.3 ± 0.18	22.4 ± 0.38	16.9 ± 0.38
9c	**18.6 ± 0.32**	21.5 ± 0.27	23.3 ± 0.65	21.4 ± 0.64	15.2 ± 0.53
Gentamycin	19.9 ± 0.18	23.4 ± 0.32	26.3 ± 0.15	24.8 ± 0.24	17.3 ± 0.12

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moiety (aryl: phenyl, **6a**; 4-fluorophenyl, **6b**; 4-bromophenyl, **6c**; 4-methylphenyl, **6d;** 4-nitrophenyl, **6e**). The effect of each substituent on benzene ring on the activity was studied. The obtained inhibition zones values for these compounds suggest that all of the evaluated hydrazone derivative possess significant antibacterial and antifungal activities against most of the tested bacteria and fungi. 4-Fluorophenyl moiety (**6b**) showed result greater than the reference drug against all the tested bacteria and fungi. The other moieties showed results near to the reference drug against most of the tested bacteria and fungi.

For search more potent antibacterial and antifungal agents, another series of hydrazide derivatives **7a–c**, which contain substituent pyrazole was synthesized. Generally, as shown in Fig. 2, a noticeable improvement in the antimicrobial activity was observed. Hydrazide derivatives **7a–c** showed potent antimicrobial activity. These activities are higher than the reference drug against all screened organisms. Changing the substituent on pyrazole from phenyl to 4-chlorophenyl to 4-methylphenyl (**7a → 7b → 7c**) had a moderate difference in the antimicrobial activity. This suggested that, the main effect may be related to the presence of the pyrazole moiety.

Table 3 Antifungal activity of the synthesized compounds and standard reference against the pathological fungi expressed as inhibition diameter zones in millimeters

Compd. no.	*A. fumigatus*	*A. clavatus*	*C. albicans*	*G. candidum*	*P. marneffei*	*S. acemosum*
2	14.6 ± 0.21	12.4 ± 0.21	11.2 ± 0.43	14.6 ± 0.31	12.8 ± 0.23	NA
3	14.5 ± 0.62	11.7 ± 0.52	10.3 ± 0.37	14.4 ± 0.73	13.5 ± 0.87	NA
5	13.5 ± 0.54	11.8 ± 0.46	10.9 ± 0.34	13.5 ± 0.74	12.2 ± 0.64	NA
6a	21.2 ± 0.33	19.2 ± 0.24	18.7 ± 0.32	22.3 ± 0.17	20.7 ± 0.58	25.2 ± 0.43
6b	**24.8 ± 0.63**	**22.5 ± 0.62**	**27.3 ± 0.65**	**26.2 ± 0.74**	**23.6 ± 0.53**	**32.7 ± 0.74**
6c	19.5 ± 0.33	18.8 ± 0.32	16.5 ± 0.44	23.5 ± 0.57	19.8 ± 0.36	25.2 ± 0.34
6d	20.4 ± 0.63	18.9 ± 0.35	21.9 ± 0.63	22.2 ± 0.51	20.2 ± 0.39	27.3 ± 0.47
6e	21.1 ± 0.37	19.1 ± 0.26	23.8 ± 0.49	21.3 ± 0.65	18.9 ± 0.32	27.8 ± 0.43
7a	**23.6 ± 0.45**	**23.9 ± 0.54**	**28.4 ± 0.87**	**27.1 ± 0.45**	**25.2 ± 0.71**	**31.1 ± 0.65**
7b	**23.9 ± 0.33**	**22.7 ± 0.29**	**28.4 ± 0.23**	**26.8 ± 0.63**	**24.9 ± 0.49**	**30.9 ± 0.29**
7c	**25.1 ± 0.65**	**24.1 ± 0.34**	**26.9 ± 0.43**	**27.9 ± 0.87**	**26.4 ± 0.56**	**34.3 ± 0.76**
8	10.9 ± 0.54	12.3 ± 0.39	NA	11.7 ± 0.52	11.7 ± 0.44	NA
9a	15.8 ± 0.24	13.2 ± 0.32	12.8 ± 0.59	11.9 ± 0.28	12.8 ± 0.26	NA
9b	19.9 ± 0.43	**20.0 ± 0.45**	23.0 ± 0.23	21.4 ± 0.59	20.2 ± 0.54	22.9 ± 0.23
9c	21.8 ± 0.32	19.2 ± 0.34	22.5 ± 0.46	21.3 ± 0.23	19.9 ± 0.65	24.8 ± 0.54
Amphotericin B	23.7 ± 0.10	21.9 ± 0.12	26.4 ± 0.20	25.4 ± 0.16	22.6 ± 0.34	30.6 ± 0.15

The meaning of the bold values is these results are promising results

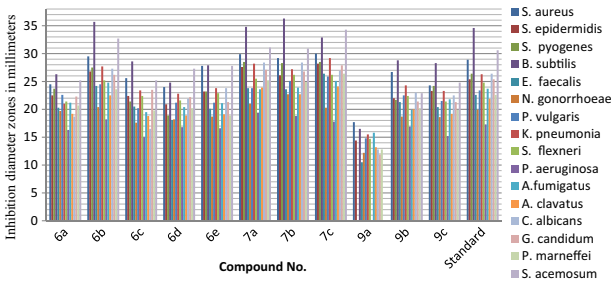

Fig. 2 Comparison of the antimicrobial activity between our synthesized hydrazone derivatives and standard antimicrobial reference drugs

The key precursor, quinaxoline derivative **3**, which is bearing *β*-propionate at N-(1) showed weak antimicrobial activity as revealed from its inhibition zones (0–15.1 mm). Changing the substituent on N-(1) from *β*-propionate to hydrazide (**3** → **8**) did not improve the antibacterial and antifungal activities. So, a series of hydrazide derivatives **9a-c** which contain various substituents at phenyl ring was synthesized. Treatment of the hydrazide derivative **8** with aromatic aldehydes improved the antibacterial and antifungal activities, where the results revealed that the antimicrobial activity of some hydrazone derivative were nearly equipotent to the reference drug. Structure **9** has at position-1 side chain ending with aryl moiety (aryl: phenyl, **9a**; 4-chlorophenyl, **9b**; 4-methylphenyl, **9c**). The effect of each substituent on benzene ring on the activity was studied. Phenyl moiety (**9a**) showed moderate antibacterial and antifungal activities while 4-chlorophenyl (**6b**) and 4-methylphenyl (**6c**) moieties showed result neat to the reference drug against most of the tested bacteria and fungi.

Minimum inhibitory concentrations against Gram-positive bacteria, Gram-negative bacteria, and fungi

The minimal inhibitory concentrations (MICs) for the promising compounds were determined using twofold serial dilution method (Abbas et al. 2013). The MICs results (μg/mL) of the most promising derivatives **6a, 6b, 6c**, **6d**, **6e, 7a, 7b, 7c, 9b**, and **9c** are presented in Table 4. The majority of synthesized compounds showed varying degrees of inhibition against the test panel of species.

Hydrazone derivatives **7a–c** showed better results when compared with the reference drugs as revealed from its MIC values. Hydrazone derivative **7c** showed fourfold potency of amphotericin B in inhibiting the growth of *Aspergilus fumigatus* (MIC 0.24 μg/mL), twofold potency of gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC 3.9 μg/mL), equipotent potency of ampicillin in inhibiting the growth of *S. pyogenes* (MIC 0.24 μg/mL), equipotent potency of gentamycin in inhibiting the growth of *P. vulgaris* (MIC 1.95 μg/mL), and *S. flexner* (MIC 0.48 μg/mL), equipotent potency of amphotericin B in inhibiting the growth of *A. clavatus* (MIC 1.95 μg/mL), *G. candidum* (MIC 0.48 μg/mL), and *P. marneffei* (MIC 1.95 μg/mL), 50% less activity compared to ampicillin against *E. faecalis* (MIC 3.90 μg/mL), 50% less activity compared to gentamycin against *P. aeruginosa* (MIC 62.5 μg/mL).

Hydrazone derivative **7b** showed twofold potency of gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC 3.9 μg/mL), equipotent potency of standard drugs in inhibiting the growth of *S. pyogenes*, *P. vulgaris*, and *G. candidum*, 50% less activity compared to ampicillin against *S. epidermidis* (MIC 0.97 μg/mL), and *E. faecalis* (MIC 3.90 μg/mL), 50% less activity compared to gentamycin

Table 4 Minimum inhibitory concentration (μg/mL) of the most potent synthesized compounds against the pathological organisms

Compd. no.	G+ve bactreia					G-ve bactreia					fungi					
	S. a.	*S.e.*	*S. p.*	*B. s.*	*E. f.*	*N. g.*	*P. v.*	*K.p.*	*S. f.*	*P. a.*	*A. f.*	*A. c.*	*C. a.*	*G. c.*	*P.m.*	*S. a.*
6a	0.97	15.63	3.9	1.95	31.25	62.5	3.9	1.95	3.9	125	3.9	15.63	31.25	1.95	3.9	0.84
6b	3.9	3.9	31.25	1.95	62.5	125	7.81	3.9	7.81	250	3.9	31.25	62.5	7.81	7.81	0.97
6c	0.24	1.95	0.48	0.06	**0.97**	15.63	3.9	0.48	1.95	62.5	**0.97**	3.9	0.48	**0.24**	3.9	0.06
6d	0.24	**0.24**	3.9	0.06	7.81	15.63	3.9	0.48	3.9	500	15.63	3.9	31.25	7.81	3.9	7.81
6e	3.9	1.95	62.5	0.97	125	500	7.81	1.95	7.81	250	7.81	15.63	31.25	7.81	15.63	0.97
7a	0.97	1.95	0.48	0.06	3.9	**7.81**	3.9	1.95	**0.48**	**31.25**	1.95	3.9	0.48	3.9	**1.95**	0.06
7b	0.24	0.97	**0.24**	0.06	3.9	**3.9**	**1.95**	0.48	3.9	62.5	1.95	3.9	0.97	**0.48**	3.9	0.06
7c	0.24	3.9	**0.24**	0.06	3.9	**3.9**	**1.95**	0.97	**0.48**	62.5	**0.24**	**1.95**	0.97	**0.48**	**1.95**	0.06
9b	3.9	62.5	31.25	125	500	62.5	7.81	3.9	1.63	250	62.5	7.81	62.5	125	125	7.81
9c	3.9	62.5	125	62.5	3.9	31.25	7.81	1.95	3.9	250	**0.97**	7.81	0.48	125	**1.95**	7.81
St	0.06	0.48	0.24	0.007	1.95	7.81	1.95	0.24	0.48	31.25	0.97	1.95	0.24	0.48	1.95	0.015

S. a. = *S. aureus*, *S. e.* = *S. epidermidis*, *S. p.* = *S. pyogenes*, *B. s.* = *B. subtilis*, *E. f.* = *E. faecalis*, *N. g.* = *N. gonorrhoeae*, *P. v.* = *P. vulgaris*, *K. p.* = *K. pneumonia*, *S. f.* = *S. flexneri*, *P. a.* = *P. aeruginosa*, *A. f.* = *A. fumigates*, *A. c.* = *A. clavatus*, *C. a.* = *C. albicans*, *G. c.* = *G. candidum*, *P. m.* = *P. marneffei*, and *S. a.* = *S. acemosum*

The meaning of the bold values is these results are promising results

against *K. pneumonia* and *P. aeruginosa*. 50% less activity compared to amphotericin B in inhibiting the growth of *A. fumigatus* (MIC 1.95 μg/mL), *A. clavatus* (MIC 3.9 μg/mL), and *P. marneffei* (MIC 3.9 μg/mL).

Hydrazone derivative **7a** showed equipotent potency of gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC 7.81 μg/mL), *S. flexner* (MIC 0.48 μg/mL), and *P. aeruginosa* (MIC 31.25 μg/mL), equipotent potency of amphotericin B in inhibiting the growth of *P. marneffei* (MIC 1.95 μg/mL). 50% less activity compared to ampicillin against *S. pyogenes* (MIC 0.48 μg/mL) and *E. faecalis* (MIC 3.90 μg/mL). 50% less activity compared to gentamycin against *P. vulgaris* and *K. pneumonia*. 50% less activity compared to amphotericin B in inhibiting the growth of *A. fumigatus* (MIC 1.95 μg/mL), *A. clavatus* (MIC 3.9 μg/mL), and *P. marneffei* (MIC 1.95 μg/mL).

Hydrazone derivative **6c** showed twofold potency of ampicillin against *E. faecalis* (MIC 0.97 μg/mL), twofold potency of amphotericin B in inhibiting the growth of *G. candidum* (MIC 0.24 μg/mL), equipotent potency of amphotericin B in inhibiting the growth of *E. faecalis* (MIC 0.97 μg/mL), 50% less activity compared to ampicillin against *S. pyogenes* (MIC 0.48 μg/mL), 50% less activity compared to gentamycin in inhibiting the growth of *N. gonorrhoeae*, *P. vulgaris, K. pneumonia*, and *P. aeruginosa*, 50% less activity compared to amphotericin B in inhibiting the growth of *A. clavatus*, *C. albicans*, and *P. marneffei*.

Hydrazone derivative **6d** showed twofold potency of ampicillin against *S. epidermidis* (MIC 0.24 μg/mL, 50% less activity gentamycin in inhibiting the growth of *N. gonorrhoeae*, *P. vulgaris*, and *K. pneumonia*, 50% less activity compared to amphotericin B in inhibiting the growth of *A. clavatus* and *P. marneffei*.

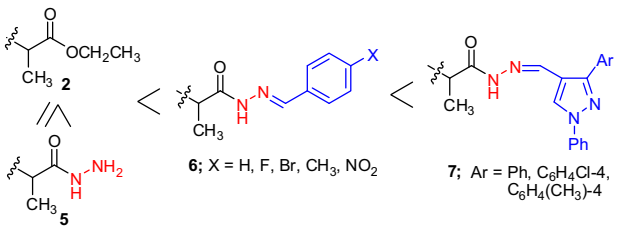

Fig. 3 Structural variations and modifications effects on the antimicrobial action of the synthesized compounds

Hydrazone derivative **9c** showed equipotent potency of amphotericin B in inhibiting the growth of *A. fumigatus* and *P. marneffei*, 50% less activity compared to ampicillin against *E. faecalis* 50% less activity compared to amphotericin B in inhibiting the growth of *C. albicans*

Conclusion

From Tables 1–4 and Fig. 2, it is clear that as shown in Fig. 3, structure–activity relationship revealed that: *β*-propionate quinaxoline derivative showed the weak antimicrobial activity. Changing the substituent from *β*-propionate to hydrazide showed little improvement in antibacterial and antifungal activities. Reaction of hydrazide derivative **5** with aldehydes improved the antibacterial and antifungal activities. Hydrazone derivatives which contain substituent pyrazole showed potent antimicrobial activity. Hydrazone derivative **7c** showed fourfold potency of amphotericin B in inhibiting the growth of *A. fumigatus*, twofold potency of gentamycin in inhibiting the growth of *N. gonorrhoeae*, equipotent potency of ampicillin in inhibiting the growth of *S. pyogenes*, equipotent potency of gentamycin in inhibiting the growth of *P. vulgaris* and *S. flexner*, equipotent potency

of amphotericin B in inhibiting the growth of *A. clavatus*, *G. candidum*, and *P. marneffei*.

Experimental section

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and may be uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a JASCO spectrophotometer. ^1H NMR spectra were recorded on Bruker spectrometers at 500 MHz. ^{13}C NMR spectra were recorded on Bruker spectrometers at 125 MHz.

Alkylation of 3-methylquinoxalin-2-ol ethyl 2-bromopropanoate

A mixture of **1** (0.01 mol), ethyl 2-bromopropanoate (0.01 mol), and anhydrous potassium carbonate (2.0 g) in acetone (50 mL) was heated under reflux for 7 h (monitored by TLC). After completion of the reaction, the mixture was filtered and the excess of acetone was evaporated under reduced pressure. The mixture of two products was purified by column chromatography on silica-gel using *n*-hexane/ethyl acetate as eluent.

Ethyl 2-(3-methylquinoxalin-2-yloxy)propanoate (2)

Yield 62%; m.p. oil; IR: ν/cm^{-1}: 2987, 2938 (CH-aliph.), 1752 (C=O), 1586 (C=N); ^1H NMR (500 MHz, $CDCl_3$): δ/ppm = 1.29 (t, 3H, $J = 7.1$ Hz, $CH_2\underline{CH_3}$), 1.75 (d, 3H, $J = 7.1$ Hz, CH_3), 2.75 (s, 3H, CH_3–quinoxaline), 4.17–4.33 (m, 2H, $\underline{CH_2}CH_3$), 5.50 (q, 1H, $J = 7.0$ Hz, CH–O), 7.52–7.65 (m, 2H, Ar–H), 7.76 (d, 1H, $J = 8.2$ Hz, Ar–H), 7.97 (d, 1H, $J = 8.1$ Hz, Ar–H); ^{13}C NMR (126 MHz, $CDCl_3$): 14.2 (CH_3), 17.5 (CH_3), 20.3 (CH_3), 61.1 (CH_2), 70.6 (CH–O), 126.7 (CH), 126.8 (CH), 128.0 (CH), 128.9 (CH), 138.9 (C), 139.4 (C), 147.8 (C), 155.1 (C=N), 171.7 (C=O); Anal. calcd. for $C_{14}H_{16}N_2O_3$ (260.29): C, 64.60; H, 6.20; N, 10.76; Found: C, 64.72; H, 6.18; N, 10.69%

Ethyl 2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)propanoate (3)

Yield 28%; m.p. oil; IR: ν/cm^{-1}: 2985, 2937 (CH-aliph.), 1744, 1659 (2C=O), 1604 (C=N); ^1H NMR (500 MHz, $CDCl_3$) δ/ppm = 1.13–1.24 (m, 3H, $CH_2\underline{CH_3}$), 1.70–1.82 (m, 3H, CH_3), 2.67 (s, 3H, CH_3–quinoxaline), 4.13–4.41 (m, 2H, $\underline{CH_2}CH_3$), 5.76 (s, 1H, CH–N), 7.20 (d, 1H, $J = 8.3$ Hz, Ar–H), 7.32–7.46 (m, 1H, Ar–H), 7.28–7.55 (m, 1H, Ar–H), 7.87 (dd, 1H, $J = 5.1, 2.9$ Hz, Ar–H); ^{13}C NMR (126 MHz, $CDCl_3$): 14.0 (CH_3), 14.1 (CH_3), 21.5 (CH_3), 51.3 (CH–N), 61.9 (CH_2), 113.6 (CH), 123.8 (CH), 129.5 (CH), 130.1 (CH), 131.6 (C), 133.2 (C), 154.4 (C), 158.3 (C=O), 169.8 (C=O); Anal. calcd. for $C_{14}H_{16}N_2O_3$ (260.29): C, 64.60; H, 6.20; N, 10.76; Found: C, 64.42; H, 6.14; N, 10.54%

2-(3-Methylquinoxalin-2-yloxy)propanoic acid (4)

A solution of **2** (38 mmol) and LiOH (38 mmol) in 15 mL of methanol and two drops of water were stirred at room temperature for 30 min. Volatiles were removed under vacuum, the solid was dissolved in water and extracted three times with ethyl acetate. The aqueous phase was adjusted to pH 3 by drop-wise addition of 5 N HCl at 0 °C. Extraction with ethyl acetate, washing of the organic phase with brine, drying using Na_2SO_4 and evaporation gave desired product in that was used as such in the next step. Yield 70%; m.p. 173–175 °C; IR: ν/cm^{-1}: 3459 (OH), 2991, 2925 (CH-aliph.), 1714 (C=O); ^1H NMR (500 MHz, MeOH) δ/ppm = 1.74 (d, 3H, $J = 7.1$ Hz, CH_3), 2.69 (s, 3H, CH_3–quinoxaline), 5.53 (q, 1H, $J = 6.8$ Hz, CH–O), 7.59 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.65 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.77 (d, 1H, $J = 8.2$ Hz, Ar–H), 7.91 (d, 1H, $J = 8.1$ Hz, Ar–H); ^{13}C NMR (126 MHz, $CDCl_3$): 16.5 (CH_3), 18.7 (CH_3), 70.4 (CH–O), 126.5 (CH), 126.7 (CH), 127.0 (CH), 129.0 (CH), 138.1 (C), 139.4 (C), 147.9 (C), 155.2 (C), 173.8 (C=O); Anal. calcd. for $C_{12}H_{12}N_2O_3$ (232.24): C, 62.06; H, 5.21; N, 12.06; Found: C, 61.89; H, 5.18; N, 11.89%

2-(3-Methylquinoxalin-2-yloxy)propanehydrazide (5)

A solution of ethyl 2-(3-methyl-2-oxoquinoxalin-1(2H)-yl) propanoate (**3**) (0.01 mol) in ethanol (20 mL) was treated with hydrazine hydrate (0.6 g, 0.012 mol). The mixture was heated under reflux for 3 h. The solid product obtained was collected by filtration and crystallized from dioxane to give the corresponding hydrazide derivative **5**; Yield 85 %; m.p. 150–152 °C; IR: ν/cm^{-1}: 3334, 3282 (NH, NH_2), 2989, 2929 (CH-aliph.), 1658 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ/ppm = 1.74 (d, 3H, $J = 6.8$ Hz, CH_3), 2.74 (s, 3H, CH_3), 5.40 (br, 2H, NH_2), 5.84 (q, 1H, $J = 6.8$ Hz, CH), 7.54–7.72 (m, 2H, Ar–H), 7.80–7.83 (m, 2H, 1Ar–H, and NH), 7.99 (d, 1H, $J = 8.0$ Hz, Ar–H); ^{13}C NMR (126 MHz, $CDCl_3$): 18.0 (CH_3), 20.4 (CH_3), 71.7 (CH-aliph.), 127.0 (CH), 127.2 (CH), 128.1 (CH), 129.3 (CH), 139.0 (C), 139.2 (C), 147.4 (C), 154.5 (C=N), 172.0 (C=O); Anal. calcd. for $C_{12}H_{14}N_4O_2$ (246.27): C, 58.53; H, 5.73; N, 22.75; Found: C, 58.63; H, 5.69; N, 22.68%

General procedure for the synthesis of thiosemicarbazone derivatives 6a–e

A mixture of hydrazide **5** (0.01 mol) and the desired aldehyde (namely benzaldehyde, 3-bromobenzaldehyde, 4-

methylbenzaldehyde, 4-fluorobenzaldehyde, or 4-nitobenzaldehyde) (0.01 mol) in dioxane (25 mL) was heated under reflux for 1 h, the solid which separated on heating was filtered and crystallized from dioxane to give the desired hydrazone derivatives **6a–e**

N′-Benzylidene-2-(3-methylquinoxalin-2-yloxy) propanehydrazide (6a)

Yield 90%; m.p. 184–186 °C; IR: ν/cm^{-1}: 3211 (NH), 2983 (CH-aliph.), 1664 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm = 1.86 (m, 3H, CH_3), 2.83 (s, 3H, CH_3), 6.33 (m, 1H, CH), 7.33–7.95 (m, 6H, 9Ar–H, and CH=N), 9.94 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 17.0 (CH_3), 20.5 (CH_3), 69.7 (CH-aliph.), 126.6 (CH), 127.0 (CH), 127.2 (CH), 127.8 (CH), 127.9 (CH), 128.6 (CH), 128.7 (CH), 128.7 (CH), 128.8 (CH), 130.3 (C), 139.0 (C), 139.2 (C), 147.4 (C), 144.5 (C=N), 173.7 (C=O); Anal. calcd. for $C_{19}H_{18}N_4O_2$ (334.37): C, 68.25; H, 5.43; N, 16.76; Found: C, 68.34; H, 5.38; N, 16.84%

N′-(4-Fluorobenzylidene)-2-(3-methylquinoxalin-2-yloxy) propanehydrazide (6b)

Yield 95 %; m.p. 179–181 °C; IR: ν/cm^{-1}: 3221 (NH), 2974, 2899 (CH-aliph.), 1671 (C=O); ^{1}H NMR (500 MHz, DMSO) δ/ppm = 1.66 (m, 3H, CH_3), 2.64 (m, 3H CH_3), 6.25 (q, 1H, J = 6.7 Hz, CH), 7.23–7.31 (m, 2H, Ar–H), 7.60–7.68 (m, 3H, Ar–H), 7.72–7.79 (m, 3H, Ar–H), 7.94 (t, 1H, J = 7.7 Hz, Ar–H), 8.08 (s, 1H, CH=N), 11.62 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 17.3 (CH_3), 20.5 (CH_3), 69.8 (CH-aliph.), 116.3 (CH), 126.9 (CH), 127.2 (CH), 127.3 (CH), 128.3 (CH), 129.4 (CH), 129.7 (CH), 131.1 (C), 138.6 (C), 143.0 (CH), 146.9 (CH), 148.4 (C), 155.3(C), 162.5 (C), 164.4 (C), 167.6 (C=N), 172.0 (C=O); Anal. calcd. for $C_{19}H_{17}FN_4O_2$ (352.36): C, 64.76; H, 4.86; N, 15.90; Found: C, 64.84; H, 4.91; N, 16.04%

N′-(4-Bromobenzylidene)-2-(3-methylquinoxalin-2-yloxy) propanehydrazide (6c)

Yield 93%; m.p. 222–224 °C; IR: ν/cm^{-1}: 3198 (NH), 2995, 2912 (CH-aliph.), 1666 (C=O); ^{1}H NMR (300 MHz, DMSO) δ/ppm = 1.66 (m, 3H, CH_3), 2.64 (m, 3H CH_3), 6.25 (q, 1H, J = 6.7 Hz, CH), 7.50–8.30 (m, 9H, 8Ar–H, and CH=N), 11.56 (br, 1H, NH); ^{13}C NMR (75 MHz, DMSO): 16.6 (CH_3), 19.9 (CH_3), 69.1 (CH-aliph.), 122.9, 126.3, 126.5, 126.6, 127.6, 128.5, 128.8, 131.6, 133.3, 138.7, 142.3, 146.3, 147.4, 154.7, 167.0 (C=N), 171.4 (C=O); Anal. calcd. for $C_{19}H_{17}BrN_4O_2$ (413.27): C, 55.22; H, 4.15; N, 13.56; Found: C, 55.10; H, 4.11; N, 13.38%

N′-(4-Methylbenzylidene)-2-(3-methylquinoxalin-2-yloxy) propanehydrazide (6d)

Yield 88%; m.p. 199–201 °C; IR: ν/cm^{-1}: 3207 (NH), 2991, 2937 (CH-aliph.), 1662 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm = 1.80–1.85 (m, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.81 (s, 3H, CH_3), 6.33–6.41 (m, 1H, CH), 7.22 (m, 2H, Ar–H), 7.49 (m, 3H, Ar–H), 7.66 (m, 2H, Ar–H), 7.91 (d, 1H, J = 8.0 Hz, Ar–H), 8.20 (s, 1H, CH=N), 9.54 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 17.0 (CH_3), 20.5 (CH_3), 21.6 (CH_3), 69.7 (CH-aliph.), 126.5 (CH), 127.2 (CH), 127.8 (CH), 127.9 (CH), 128.7 (CH), 129.5 (CH), 130.7 (CH), 138.8 (C), 139.5 (C), 140.7 (C), 144.4 (CH), 147.9 (C), 149.5 (CH), 155.37 (C), 167.2 (C), 173.3 (C=O); Anal. calcd for $C_{20}H_{20}N_4O_2$ (348.40): C, 68.95; H, 5.79; N, 16.08; Found: C, 69.12; H, 5.81; N, 16.17%

2-(3-Methylquinoxalin-2-yloxy)-*N*′-(4-nitrobenzylidene) propanehydrazide (6e)

Yield 95%; m.p. > 300 °C; IR: ν/cm^{-1}: 3203 (NH), 1663 (C=O); ^{1}H NMR (300 MHz, DMSO) δ/ppm = 1.67 (m, 3H, CH_3), 2.60 (m, 3H CH_3), 6.26 (q, 1H, J = 6.7 Hz, CH), 7.53–8.40 (m, 9H, 8Ar–H, and CH=N), 11.85 (br, 1H, NH); ^{13}C NMR (75 MHz, DMSO): 16.6 (CH_3), 19.9 (CH_3), 69.1 (CH-aliph.), 123.8, 126.3, 126.5, 126.7, 127.5, 127.7, 129.0, 138.1, 138.6, 140.3, 141.3, 145.1, 147.4, 154.7, 167.4 (C=N), 171.7 (C=O); Anal. calcd. for $C_{19}H_{17}N_5O_4$ (379.37): C, 60.15; H, 4.52; N, 18.46; Found: C, 60.34; H, 4.48; N, 18.31%

General procedure for the condensation of the hydrazide 5 with formylpyrazole

To a solution of the hydrazide **5** (0.01 mol) in dioxane (30 mL), formylpyrazole (0.01 mol) was added and the reaction mixture was heated under reflux for 3 h. The solid obtained upon cooling was collected by filtration, dried, and recrystallized from dioxane.

N′-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)-2-(3-methylquinoxalin-2-yloxy)propanehydrazide (7a)

Yield 80%; IR: ν/cm^{-1}: 3226 (NH), 1678 (C=O); ^{1}H NMR (300 MHz, DMSO) δ/ppm = 1.61 (m, 3H, CH_3), 2.63 (m, 3H CH_3), 6.15 (q, 1H, J = 6.7 Hz, CH), 7.34–8.92 (m, 16H, 15Ar–H, and CH=N), 11.54 (br, 1H, NH); ^{13}C NMR (75 MHz, DMSO): 16.8 (CH_3), 19.9 (CH_3), 69.3 (CH-aliph.), 116.6, 118.7, 126.4, 126.6, 126.7, 126.9, 127.1, 127.7, 128.1, 128.4, 128.7, 129.1, 129.5, 131.9, 132.3, 136.7, 138.0, 138.7, 138.9, 140.7, 147.8, 151.8, 154.8, 166.5 (C=N), 171.1 (C=O); Anal. calcd. for $C_{28}H_{24}N_6O_2$ (476.53): C, 70.57; H, 5.08; N, 17.64; Found: C, 70.64; H, 5.11; N, 17.78%

N′-((3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(3-methylquinoxalin-2-yloxy) propanehydrazide (7b)

Yield 85%; IR: ν/cm^{-1}: 3201 (NH), 1676 (C=O); ^1H NMR (300 MHz, DMSO) δ/ppm = 1.63 (m, 3H, CH_3), 2.63 (m, 3H CH_3), 6.15 (q, 1H, CH), 7.30–8.92 (m, 15H, 14Ar–H, and CH=N), 11.50 (br, 1H, NH); ^{13}C NMR (75 MHz, DMSO): 16.7 (CH_3), 19.8 (CH_3), 69.3 (CH-aliph.), 116.8, 118.6, 126.3, 126.5, 126.7, 126.9, 127.6, 128.3, 128.5, 128.9, 129.4, 130.0, 136.4, 138.0, 138.8, 140.4, 154.7, 158.6 (C=N), 171.0 (C=O); Anal. calcd. for $C_{28}H_{23}ClN_6O_2$ (510.97): C, 65.82; H, 4.54; N, 16.45; Found: C, 65.78; H, 4.51; N, 16.38%

2-(3-Methylquinoxalin-2-yloxy)-*N*′-((1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methylene)propanehydrazide (7c)

Yield 80%; IR: ν/cm^{-1}: 3199 (NH), 1665 (C=O); ^1H NMR (300 MHz, DMSO) δ/ppm = 1.61 (m, 3H, CH_3), 2.32 (m, 3H CH_3), 2.63 (m, 3H CH_3), 6.17 (q, 1H, $J = 6.7$ Hz, CH), 7.25–8.90 (m, 15H, 14Ar–H, and CH=N), 11.49 (br, 1H, NH); ^{13}C NMR (75 MHz, DMSO): 16.8 (CH_3), 19.9 (CH_3), 20.7 (CH_3), 69.2 (CH-aliph.), 118.6, 120.3, 126.3, 126.7, 127.0, 127.6, 128.2, 128.9, 129.0, 129.1, 129.4, 136.7, 137.9, 138.0, 138.7, 138.9, 140.9, 147.5, 154.9, 166.4 (C=N), 171.0 (C=O); Anal. calcd. for $C_{29}H_{26}N_6O_2$ (490.56): C, 71.00; H, 5.34; N, 17.13; Found: C, 70.89; H, 5.29; N, 16.89%

2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)propanehydrazide (8)

Yield 84%; m.p. 98–100 °C;IR: ν/cm^{-1}: 3456, 3354, 3264 (NH, NH_2), 1663, 1652 (C=O); ^1H NMR (500 MHz, DMSO) δ/ppm = 1.56 (d, 3H, $J = 6.8$ Hz, CH_3), 2.46 (s, 3H, CH_3), 4.25 (br, 2H, NH_2), 5.85 (s, 1H, CH), 7.24 (s, 1H, Ar–H), 7.35 (t, 1H, $J = 7.3$ Hz, Ar–H), 7.52 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.77 (d, 1H, $J = 7.6$ Hz, Ar–H), 9.17 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 14.1 (CH_3), 21.8 (CH_3), 51.4 (CH-aliph.), 115.2 (CH), 123.6 (CH), 129.6 (2CH), 133.2 (C), 154.9 (C), 158.4 (C), 168.6 (2C=O); Anal. calcd. for $C_{12}H_{14}N_4O_2$ (246.27): C, 58.53; H, 5.73; N, 22.75; Found: C, 58.71; H, 5.67; N, 22.64%

N′-Benzylidene-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl) propanehydrazide (9a)

Yield 80%; m.p. 178–180 °C; IR: ν/cm^{-1}: 3210 (NH), 2995, 2912 (CH-aliph.), 1666 (C=O); ^1H NMR: (500 MHz, CD_2Cl_2) δ/ppm = 1.63–1.81 (m, 3H, CH_3). 2.58 (d, 3H, $J = 28.4$ Hz, CH_3), 6.09 (s, 1H, CH), 6.99–7.81 (m, 10H, Ar–H, and CH=N), 9.12 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 13.6 (CH_3), 21.5 (CH_3), 51.0 (CH-aliph.), 114.0, 123.7, 127.2 (2C), 128.7 (3C), 129.7, 130.1 (2C), 130.3, 133.0, 133.2, 144.5, 154.2 (C=O), 158.1(C=O); Anal. calcd. for $C_{19}H_{18}N_4O_2$ (334.37): C, 68.25; H, 5.43; N, 16.76; Found: C, 68.36; H, 5.39; N, 16.57%

N′-(4-Chlorobenzylidene)-2-(3-methyl-2-oxoquinoxalin-1 (2H)-yl)propanehydrazide (9b)

Yield 90%; m.p. 263–265 °C; IR: ν/cm^{-1}: IR: ν/cm^{-1}: 3220 (NH), 2995 (CH-aliph.), 1670, 1654 (C=O); ^1H NMR: (500 MHz, DMSO) δ/ppm = 1.57 (d, 3H, $J = 6.5$ Hz, CH_3), 2.52 (s, 3H, CH_3), 6.03 (m, 1H, CH), 6.73–8.15 (m, 9H, Ar–H, and CH=N), 11.51 (br, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): 13.9 (CH_3), 19.1 (CH_3), 51.3 (CH-aliph.), 114.8, 124.0, 128.4, 128.9 (2C), 129.1, 129.4, 129.7, 130.0 (2C), 130.3, 133.1, 133.2, 134.4, 153.9, 158.0 (C=O); Anal. calcd. for $C_{19}H_{17}ClN_4O_2$ (368.82): C, 61.87; H, 4.65; N, 15.19; Found: C, 61.93; H, 4.61; N, 15.07%

2-(3-Methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*′-(4-methylbenzylidene)propanehydrazide (9c)

Yield 77%; m.p. 233–235 °C; IR: ν/cm^{-1}: 3231 (NH), 2985, 2897 (CH-aliph.), 1666 (C=O); ^1H NMR: (500 MHz, CD_2Cl_2) δ/ppm = 1.66 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.09 (s, 1H, CH), 6.63–7.80 (m, 9H, Ar–H, and CH=N), 8.94 (br, 1H, NH); ^{13}C NMR (126 MHz, CD_2Cl_2): 13.6 (CH_3), 21.5 (CH_3), 21.6 (CH_3), 51.0 (CH-aliph.), 114.0, 123.6, 127.2 (2C), 129.4 (3C), 129.7, 130.0, 130.1, 132.0, 133.3, 140.7, 144.6, 154.3 (C=O), 158.1 (C=O); Anal. calcd. for $C_{20}H_{20}N_4O_2$ (348.40): C, 68.95; H, 5.79; N, 16.08; Found: C, 68.78; H, 5.71; N, 16.14%

Antimicrobial evaluation

The antimicrobial screening and MICs of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

The antimicrobial screening

The discs of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper discs impregnated with a solution of the test compound in DMF (100 mL, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 106 CFU/mL (colony forming units/mL) and 104 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two discs of filter paper were inoculated in each plate. DMF alone was used as control at the same above-

mentioned concentration and due to this there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h at 25 °C for fungi. The mean zone of inhibition was measured in mm standard deviation on a range of environmental and clinically pathogenic microorganisms. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

MIC measurement

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, Ampicillin, Gentamycin and Amphotricin B were prepared in DMF at concentrations 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500–0.007 µg/mL. 10 mL of the broth containing about 106 CFU/mL of test bacteria was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the MIC values were recorded as the lowest concentrations of the substance that inhibited the growth of the tested organisms judged by the absence of visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions. The experiment was carried out in triplicate and the average was calculated.

Compliance with ethical standards

Conflicts of interest The authors declare that there is no conflict of interest.

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