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Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives

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ABSTRACT

A simple and efficient method has been developed for the synthesis of various 2-quinoxalinone-3-hydrazone derivatives using microwave irradiation technique. The series of 2-quinoxalinone-3-hydrazone derivatives synthesized, were structurally confirmed by analytical and spectral data and evaluated for their antimicrobial activities. The results showed that this skeletal framework exhibited marked potency as antimicrobial agents. The most active antibacterial agent was 3-{2-[1-(6-chloro-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1*H*)-one, **7** while 3-[2-(propan-2-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, **2** appeared to be the most active antifungal agent.

1. Introduction

Day by day, the chemistry of carbon–nitrogen double bond of hydrazone is fast becoming the backbone of condensation reaction in benzo-fused N-heterocycles.[1] Hydrazone containing azomethine –NHN=CH protons constitute an important class of compounds for new drug development.[2] Many researchers have synthesized these compounds[2] as target structures and evaluated their biological activities. Hydrazones have been reported to possess, among others, antimicrobial,[3] antitubercular,[4,5] anticonvulsant,[6] analgesic,[7] anti-inflammatory,[8,9] antiplatelet,[10] anticancer,[11,12] antifungal,[13] antiviral,[14] antitumoral,[15,16] antibacterial[17] and antimalarial[18] activities. Reactions of phenylhydrazine, the first hydrazine derivative characterized by Fischer in 1875,[19] with isatin,[20–22] coumarin-3-carboxylate,[23] 2-bromobenzaldehyde,[24] 4-azido-3-acyl-2-quinolone,[25] mercaptoacetic acid,[26] and 1,2-benzothiazole derivatives[27] were reported to afford the corresponding hydrazones in moderate to high yields. Hydrazone has been recently established as a good precursor for one pot synthesis of C-4 functionalized 1,2,3,4-tetrahydroquinolones containing a quaternary stereocenter.[28] Diflunisal hydrazide-hydrazone was also prepared as possible dual acting antimicrobial/antituberculosis agents with anti-inflammatory properties.[29] The upsurge of widespread multi-drug resistance microorganisms such as *Staphylococcus epidermidis*,[30,31] *Staphylococcus aureus*,[32,33] *Pseudomonas aeruginosa*,[34,35] *Escherichia coli*,[36,37] *Enterococcus faecium*,[38,39] *Scedosporium apiospernum*,[40] among others, had been reported[41] as a major threat to human health. In view of this occurrence of microorganisms' resistance to drugs[42] currently in use and emergence of new diseases,[43] there is a continuous need for the synthesis of new organic compounds as potential antimicrobial agents using a fast and efficient approach. Thus, it is conceivable to develop a series of quinoxalinylhydrazones by microwave assisted approach with the aim of investigating their antimicrobial properties.

2. Results and discussion

2.1. Chemistry

In the continuation of our effort on the quinoxaline moieties,[44] the hydrazone derivatives were synthesized in order to evaluate their antimicrobial activity. Firstly, the 1,2,3,4-tetrahydroquinoxaline-2,3-dione earlier synthesized by the modified procedure of Obafemi and Pfeiferer,[45] was made to react with hydrazine hydrate according to a known procedure[46] to afford 3-hydrazinoquinoxalin-2(1*H*)-one, **1** which is the main precursor in this present study (Scheme 1). The treatment of **1** with some acyclic ketones under microwave irradiation afforded the hydrazones **2–4**. The preliminary structure activity relationship studies were performed by varying the hydrazone side chain in order to determine how these subunits affect the antibacterial activity of the entire template. Based on these, we made related modification to the benzofused side chain of 3-acetylcoumarin and isatin by changing the substituents at 6- and 5-positions, respectively before condensing them

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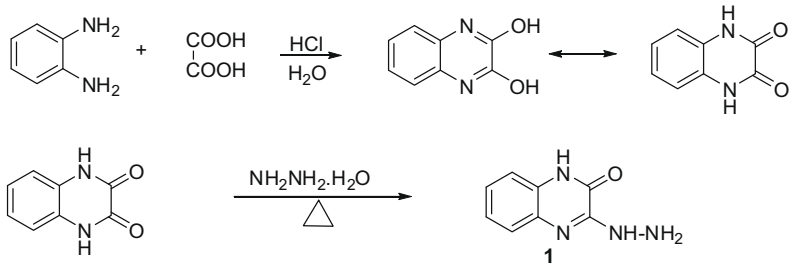


Scheme 1. Synthesis of 3-hydrazinoquinoxalin-2(1*H*)-one, **1**.

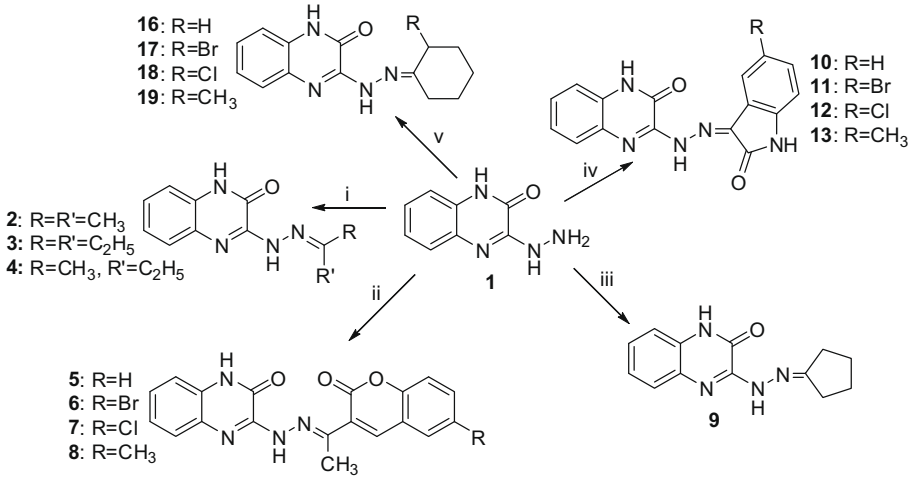
with 3-hydrazinoquinoxalin-2(1*H*)-one, **1** precursor. Hence, the condensation of **1** with 6-substituted acetylcoumarin and 5-substituted isatin (R = H, Br, Cl, CH_3) led to the formation of hydrazones **5–8** and **10–13**, respectively (Scheme 2). The preparation of 3-[2-(2-substitutedcyclohexylidene)hydrazinyl]quinoxalin-2(1*H*)-one **17–19** was achieved by microwave irradiation of an equimolar amount of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** and 2-substituted cyclohexanone (R = H, Br, Cl, CH_3). Rapid treatment of **1** with cyclic ketones; cyclopentanone, anthrone and camphor produced 2-quinoxalinone-3-hydrazone derivatives **9, 14** and **15**, respectively.

Generally speaking, the infrared spectra of the compounds **(1–19)** showed absorption bands due to the stretching vibrations of N–H, C=O, C=C and C=N at 3412–3118 cm^{-1}, 1742–1648 cm^{-1}, 1620–1600 cm^{-1} and 1588–1531 cm^{-1}, respectively while the electronic transition in UV–vis spectra of **1–19** gave rise to wavelength (λ_{max}) ranging from 210 nm to 425 nm. Compound **2** was used as a representative for the spectroscopic study of the hyrazone templates. Hence, in the infrared spectrum of **2** for instance, the stretching vibrational frequencies of N–H and C=O appeared at 3241 cm^{-1} and 1685 cm^{-1}, respectively while the absorption band of CH aliphatic occurred at 2928 cm^{-1}. The C=C of aromatic and C=N of hydrazone were responsible for the band at 1612 cm^{-1} and 1563 cm^{-1}, respectively. Similarly, The UV–vis absorption spectrum of **2** showed a peak at λ_{max} = 216 nm (log ε_{max} = 3.76) which was the value for the confirmation of presence of phenyl ring due to $\pi \rightarrow \pi^*$ transition while the bathochromic shift observed at λ_{max} = 304 nm (log ε_{max} = 3.23) was due to the presence of C=N of hydrazone; a noticeable peak were also seen at λ_{max} = 348 nm (log ε_{max} = 3.03) while a shoulder was observed at λ_{max} = 330 nm (log ε_{max} = 3.13 s).

In addition, the chemical shift and multiplicity patterns correlated well with the proposed structures. Thus, the ^{1}H NMR of **2** showed a singlet corresponding to resonance of N–H of hydrazone at δ 6.13 while that of amide was observed at δ 13.55 downfield of TMS scale and it was exchangeable with D_2O. The multiplet at δ 7.37–7.83 confirmed the presence of four aromatic protons. Two highly intense sharp singlets at δ 2.28 and 2.39 established the presence of six alkyl protons of two -CH_3 substituents attached to the hydrazone carbon. The ^{13}C NMR of **2** revealed eleven carbon atoms with C=O having highest signal at 158.0 ppm while the two –CH_3 carbon atoms appeared lowest signals at 25.0 ppm and 19.0 ppm. The remaining eight carbon atoms were sp^2 hybridized with their signals ranging between 157.6 and 115.2 ppm. The mass spectral data of **2** showed molecular ion peak at *m/z* 216 (25%) which was in agreement with the molecular mass of the compound ($C_{11}H_{12}N_4O$) while base peak was observed at *m/z* 160 (100%). Other peaks appeared at *m/z* 146, and 76 with relative intensities of 82% and 12%, respectively due to some fragmentation processes. The result of elemental analysis did not only correlate well with the molecular masses of all compounds but also showed a consistent minimum difference of not more than ±0.39 between % calculated and % found for the carbon, hydrogen and nitrogen of compound **2** as well as other compounds.

2.2. Antibacterial activities

The antimicrobial sensitivity testing of the nineteen synthesized compounds were assayed using agar diffusion technique against the test organisms (clinical isolates). Prior to the assay,



(i) Acyclic ketone (ii) 6-substituted-3-acetylcoumarin (iii) cyclopentanone (iv) 5-substituted isatin
(v) 2-substituted cyclohexanone. Reaction Condition: Microwave Irradiation (MWI) at 140ºC

Scheme 2. Synthesis of some 2-quinoxalinone-3-hydrazone derivatives.

Table 1
Results of antimicrobial screening on gram positive and negative bacteria with zones of inhibition in mm

Compd No.	*B. a*	*B. s*	*B.su*	*B. c*	*S. a*	*E. c*	*P. f*	*K. p*	*S. d*	*P. a*	*C.a*
1	24	15	20	28	24	25	0	10	22	15	18
2	14	30	30	24	12	30	0	12	13	0	31
3	0	25	20	28	10	15	0	15	0	0	18
4	12	17	16	25	19	21	0	19	0	0	22
5	18	28	32	20	15	30	0	20	0	0	29
6	16	29	30	15	14	18	0	18	0	0	21
7	25	30	33	30	32	30	10	32	19	14	14
8	14	15	16	25	19	15	0	19	12	10	10
9	10	14	12	20	0	17	10	14	12	0	29
10	11	13	14	0	19	10	0	12	0	17	20
11	8	22	13	15	20	13	11	15	11	0	15
12	20	24	20	28	25	19	0	20	10	0	18
13	0	13	15	10	19	14	0	17	14	0	30
14	14	18	12	25	14	20	0	21	0	15	9
15	5	14	16	10	11	18	0	16	0	13	21
16	0	15	16	25	19	11	12	17	10	0	21
17	19	13	20	28	13	13	0	12	15	0	19
18	14	14	16	12	12	12	9	13	0	0	15
19	16	25	12	13	19	12	13	15	0	0	14
Str.	18	23	20	28	21	0	30	0	22	0	NA
Nys.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	29

B. a = *Bacillus anthracis* (LIO)$^{G+}$, *B. s* = *Bacillus stearothermophilus* (NCIB 8222)$^{G+}$, *B. su* = *Bacillus subtilis* (NCIB 3610)$^{G+}$, *B. c* = *Bacillus cereus* (NCIB 6349)$^{G+}$, *S. a* = *Staphylococcus aureus* (NCIB 8588)$^{G+}$, *E. c* = *Escherichia coli* (NCIB 86)$^{G-}$, *P. f* = *Pseudomonas fluorescence* (NCIB 3756)$^{G-}$, *K. p* = *Klebsiella pneumonia* (NCIB 418)$^{G-}$, *S. d* = *S higella dysenteriae* (LIO)$^{G-}$, *Candid albican* (LIO), *P. a* = *Pseudomonas aeruginosa* (NCIB 950), G+ = Gram positive, *G– = Gram negative*, NA = not applicable, Str. = Streptomycin, Nys. = Nystatin.

the clinical isolates were tested for viability by resuscitating the organisms in buffered peptone broth, after which it was sub-cultured into nutrient agar medium and incubated at 37 °C for 24 h. The isolates were sub-cultured in a nutrient broth at 37 °C for 8 h prior to antibacterial testing. The Mueller Hinton agar was inoculated with the test organisms and solutions of the different synthesized compounds dissolved in DMSO were aseptically introduced into the bored holes. Streptomycin and Nystatin were used as reference clinical standards for the antibacterial and antifungal activities, respectively. The clinical isolates used include five gram positive bacteria; *Bacillus anthracis* (LIO), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus subtilis* (NCIB 3610), *Bacillus cereus* (NCIB 6349), *S. aureus* (NCIB 8588) and five gram negative bacteria; *E. coli* (NCIB 86), *Pseudomonas fluorescence* (NCIB 3756), *Klebsiella pneumonia* (NCIB 418), *Shigella dysenteriae* (LIO), *P. aeruginosa* (NCIB 950). The results of their antibacterial sensitivity testing measured in mm were reported in Table 1. The result of antifungal properties of these synthesized compounds on *Candida albicans* (LIO) was represented in histogram as shown in Figure 1.

The choice of use of Streptomycin as clinical standards is based on the fact that at low concentrations, Streptomycin only inhibits growth of the bacteria through induction of prokaryotic ribosomes to misread mRNA.[47] Streptomycin also prevents initiation of protein synthesis and leads to death of microbial cells. Also in humans, they have structurally different ribosomes from bacteria, thereby allowing the selectivity of this antibiotic for bacteria. The choice of Nystatin is that is a polyene antifungal antibiotic which is both fungistatic and fungicidal against a wide variety of yeasts. From the antibacterial screening, compounds **3, 9, 10, 13** and **16** showed activities on most of the five gram positive bacterial strains except *B. anthracis*, (**3, 13, 16**), *S. aureus* (**9**), *B. cereus* (**10**) while other synthesized compounds as well as Streptomycin standard were active on all the gram positive bacteria with zones of inhibition ranging from 5 mm to 33 mm (Table 1). Compared to Streptomycin, compounds **1, 7** and **12** showed larger zones of inhibition against *B. anthracis* (i.e., >18 mm) and *S. aureus* (i.e., >21 mm) while **1, 3, 12** and **17** showed nearly similar zones of inhibition as Streptomycin upon *B. subtilis* (20 mm) and *B. cereus* (28 mm). The result of antimicrobial screening (sensitivity testing) on five gram negative is also shown in Table 1. As a general observation, *E. coli* and *K. pneumoniae* developed resistance against Streptomycin whereas all the synthesized compounds **1–19** were active on these two organisms with the zones of inhibition ranging from 10 mm to 32 mm with **7** showing largest zone of inhibition (32 mm).

These significant antibacterial activities of the synthesized compounds may be explained with clue of the site of action of hydrazones, where it interacts with bases of DNA of the organisms, and thus inserts (intercalates) between the stacked bases of helix. This insertion possibly causes a stretching of the DNA duplex and the DNA polymerase is fooled into inserting an extra base opposite an intercalated molecule thereby results to frame shifts. The frame shifts invariably will affect the physiological activity by not arraying the right bases that confer resistance on the organisms. Thus, the organism becomes susceptible to the influence of the synthesized compounds. On the other hand, most of the synthesized

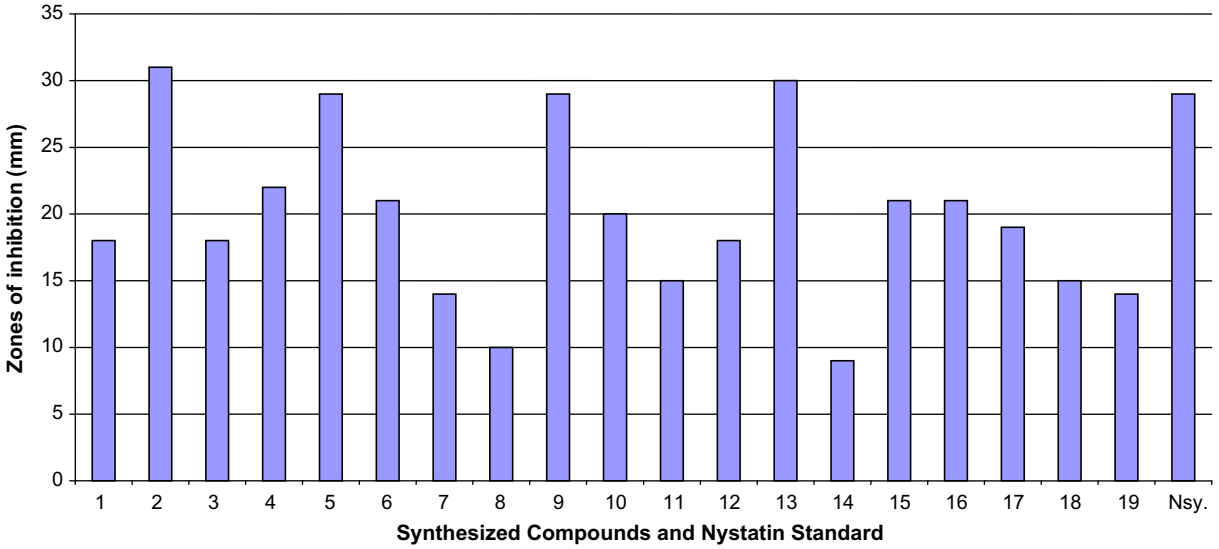


Figure 1. Result of the screening on fungus, *Candida albican*.

Table 2
Results of minimum inhibitory concentrations (MIC) on some selected bacteria in μg/ml

Compd No.	*B. anthracis*	*B. stearotherm.*	*B. subtilis*	*B. cereus*	*S. Aureus*	*E. coli*	*K. pneumonia*	*S. dysenteriea*
1	15.6	15.6	15.6	15.6	125	15.6	125	125
2	62.5	7.8	7.8	15.6	62.5	7.8	15.6	31.3
3	R	7.8	31.3	7.8	62.5	31.3	31.3	R
4	62.5	31.3	31.3	7.8	15.6	31.3	62.5	R
5	62.5	31.3	7.8	31.3	31.3	31.3	31.3	R
6	62.5	7.8	7.8	62.5	62.5	62.5	62.5	R
7	7.8	7.8	7.8	7.8	15.6	7.8	7.8	15.6
8	62.5	31.3	31.3	7.8	15.6	62.5	31.3	62.5
9	125	62.5	31.3	15.6	R	31.3	15.6	62.5
10	125	62.5	62.5	R	31.3	125	62.5	15.6
11	125	15.6	62.5	62.5	15.6	62.5	31.3	125
12	15.6	7.8	15.6	7.8	7.8	15.6	7.8	62.5
13	R	62.5	31.3	62.5	15.6	31.3	15.6	31.3
14	31.3	15.6	31.3	7.8	15.6	15.6	7.8	R
15	125	31.3	15.6	62.5	62.5	15.6	31.3	R
16	R	31.3	31.3	15.6	15.6	62.5	15.6	125
17	15.6	31.3	7.8	7.8	31.3	31.3	31.3	15.6
18	31.3	31.3	15.6	31.3	62.5	62.5	31.3	R
19	15.6	7.8	62.5	15.6	7.8	31.3	7.8	R
Str.	31.3	7.8	15.6	7.8	7.8	R	R	15.6

R = resistance.

hydrazone compounds showed either low or no zones of inhibition against *P. fluorescence* and *S. dysenteriae* while Streptomycin showed high activity on these two organisms with large zone of inhibition of 30 mm and 22 mm, respectively. From the result, it indicated that though Streptomycin had broad spectrum of activity, the synthesized compounds (i.e., the synthesized hydrazones **2–19** and the precursor **1** also showed reasonable level of activity, most especially on *E. coli* and *K. pneumoniae*. Large zones of inhibition and broad spectrum of activities were observed for all the tested organisms except *P. fluorescence* and *P. aeruginosa* which were sensitive to only six out of 19 compounds screened. The little or no zones of inhibition exhibited by *P. fluorescence, P. aeruginosa* and *S. dysenteriae* may be explained by the hardy cell wall of the organisms which contains porins and efflux pumps called ABC transporters, which pump out some antibiotics before they are able to act. It may also be due to the protective biofilms formed by these organisms.[48]

Furthermore, minimum inhibitory concentration (MIC) test was carried out on eight organisms (five gram positive and three gram negative bacteria) using Russell and Furr method (1977).[49] The result is shown in Table 2. The MIC values of **1**, **9**, **10**, **11**, **15** varied between 15.6 μg/ml and 125 μg/ml for all bacterial strains and also for **13, 16, 18**, the MIC values varied between 15.6 μg/ml and 62.5 μg/ml; for **2, 3, 4, 5, 6, 8, 12, 19** the value varied between 7.8 μg/ml and 62.5 μg/ml and between 7.8 μg/ml and 31.3 μg/ml for **14, 17** and the Streptomycin while **7** stood out by inhibiting the growth of the organisms at the lowest concentration ranging between 7.8 μg/ml and 15.6 μg/ml. This result indicated that **7** had the highest activity while **12** and **19** competed favorably with the Streptomycin. From the overview of the SAR study, it was discovered that the nature of substitutent on 5-position and 6-positions of the phenyl group of isatin and coumarin moieties, respectively had positive effect on the antibacterial activity in which the order include Cl > Br > CH_3 > H. In like manner, all the compounds were screened for their in vitro antifungal activity against *C. albican* with the zone of inhibition duly reported in mm (Fig. 1) using a standard procedure.[50] Upon the inoculation of *C. albican* with the solution of compound **5, 9** and Nystatin, the observed zone of inhibition was 29 mm while better growth inhibition was noticed when compounds **2** and **14** were used and the zones of inhibition were 31 mm and 30 mm, respectively. However, all other compounds displayed lower activity compared to Nystatin because of lower zone of inhibition in the range between 9 mm and 22 mm. From this comparative study, compound **2** emerged as the most active antifungal agent.

3. Conclusion

It was discovered that microwave assisted approach is highly efficient procedure for the preparation of various 2(1*H*)-quinoxalinone-3-hydrazone derivatives in moderate to excellent yield. The reactions occurred remarkably fast, under mild condition using highly inexpensive reagents and microwave oven as the irradiation source. 3-{2-[1-(6-Chloro-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl} quinoxalin-2(1*H*)-one, **7**, emerged as the most active antibacterial agent while 3-[2-(propan-2-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, **2** appeared to be the most active antifungal agent upon its action on *C. albican*. Thus, this work will be very useful for further studies in terms of toxicity effect and Structural Activity Relationship (SAR) to improve their biological and pharmacological properties.

4. Experimental

4.1. General conditions

Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. Infra red spectra were recorded as KBr pellets on a Buck Spectrometer while UV–vis spectra were recorded on a He-λiosα UV–vis Spectrometer v2.05. The ^{1}H and ^{13}C NMR were run on a Jeol-EX 300 spectrometer (δ in ppm relative to Me_4Si). Mass spectra were run on Finnigan MAT 312 machine. All compounds were routinely checked by TLC on silical gel G plates using acetone/benzene (9:1, v/v) solvent system and the developed plates were visualized by UV light. The elemental analysis (C, H, N) of compounds were performed using a Carlo Erba-1108 elemental analyzer. Results were found to be in good agreement with the calculated values (Table 1). The microwave assisted syntheses were carried out using a CEM Discover monomode oven operating at 2450 MHz monitored by a PC computer and temperature maintained at a constant value (140 °C) within the power modulation of 300 W. Stirring was provided by an in situ magnetic stirrer while reactions were performed in open glass vessels within a ramp time of 75 s to 3 min. All reagents used were obtained from Sigma–Aldrich Chemicals, except acetone, ethanol, oxalic acid and vanillin which were obtained from BDH Chemical Limited. Solvents used

were of analytical grade and, when necessary, were purified and dried by standard methods.

4.2. Synthesis

4.2.1. 3-Hydrazinoquinoxalin-2(1*H*)-one, 1

To a solution of 1,2,3,4-tetrahydroquinoxaline-2,3-dione (20.1 g, 124.0 mmol) in hydrazine hydrate (100.0 ml, 2.2 mol), water (50 ml) was added and the resulting mixture was refluxed for 3 h. The mixture was allowed to cool and the formed precipitate was filtered, recrystallized from ethanol to give **1** 19.8 g (90%) as a yellow solid: mp >360 °C, (mp >360 °C, Lit.).[46] IR (cm^{-1}, KBr): ν_{max} 3412(N–H), 3280(N–H), 3175(N–H), 1679(C=O), 1620(C=C). λ_{max} (log ε_{max}): 216(4.34), 247(3.75s), 327(3.61s). ^1H NMR (300 MHz, DMSO-d_6): δ 5.81(s-br, 2H, NH_2; D_2O exchangeable), 7.49–7.96(m, 4H, Ar-H), 8.14(s, 1H, NH; D_2O exchangeable), 12.55(s, 1H, NH; D_2O exchangeable). ^{13}C NMR (300 MHz, DMSO-d_6): δ 190.5(C=O), 141.9, 134.2, 125.7, 119.6, 117.0, 115.4, 110.4 ppm. MS: in *m/z*[rel.%]: 176[55.5%], 161[92.3%], 146[85.5%], 118[100%], 106[80.1%], 78[40.5%]. Anal. Calcd for $C_8H_8N_4O$: C, 54.55; H, 4.55; N, 31.82. Found: C, 54.52; H, 4.57; N, 31.83.

4.2.2. General procedure of 3-[2-(alkylidene)hydrazinyl]quinoxalin-2(1*H*)-one 2–4

To a mixture of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) and acyclic ketones (5.7 mmol), was added ethanol (20 ml). The reaction mixture was taken in round-bottomed flask placed in a microwave oven and irradiated at 400 W for 3 min and then the solvent was removed by vacuum distillation. The solid product was filtered, dried and recrystallized from ethanol to afford 3-[2-(alkylidene)hydrazinyl]quinoxalin-2(1*H*)-one **2–4**.

4.2.2.1. 3-[2-(Propan-2-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 2. Reagents: Compound **1** (1.0 g, 5.7 mmol), acetone (0.42 ml, 5.7 mmol), ethanol (20 ml). Yield 0.8 g (68.3%) as a creamy solid: mp 256 °C (sharp). IR (cm^{-1}, KBr): ν_{max} 3241(N–H), 2928(CH aliphatic), 1685(C=O), 1612(C=C), 1563(C=N). λ_{max} (log ε_{max}): 216(3.76), 304(3.23), 330(3.13s), 348(3.03). ^1H NMR [300 MHz, CH_3OH-d_4]: 2.28(s, 3H, CH_3), 2.39(s, 3H, CH_3), 6.13(s, 1H, NH, D_2O exchangeable), 7.37–7.83(m, 4H, Ar-H), 13.55(s-br, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.0(C=O), 157.6, 151.4, 142.7, 131.7, 125.9, 123.5, 119.0, 115.2, 25.0, 19.0. MS: in *m/z*[rel.%]: 216[M^+, 25%], 160[100%], 146[82%], 76[12%]. Anal. Calcd for $C_{11}H_{12}N_4O$: C, 61.11; H, 5.56; N, 25.93. Found: C, 61.40; H, 5.53; N, 25.90.

4.2.2.2. 3-[2-(Pentan-3-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 3. Reagents: Compound **1** (1.0 g, 5.7 mmol), pentan-3-one (0.60 ml, 5.7 mmol), ethanol (20 ml). Yield 0.79 g (56.8%). Mp 277–278 °C. IR (cm^{-1}, KBr): $\nu\nu_{max}$ 3270(N–H), 1675(C=O), 1600(C=C), 1570(C=N), 1270(m). λ_{max} (log ε_{max}): 220(3.71), 345(3.41s). ^1H NMR [300 MHz, CH_3OH-d_4]: 0.86(t, 6H, 2 × CH_3), 1.57(q, 4H, 2 × CH_2), 7.00(s, 1H, NH, D_2O exchangeable), 7.09–8.28(m, 4H, Ar-H), 8.00(s-br, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 163.4(C=O), 158.0, 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 24.4(CH_2), 18.4(CH_2), 11.4(CH_3), 11.4(CH_3). MS: in *m/z*[rel.%]: 244[M^+, 10%], 188[65%], 161[100%], 146[85%], 118[75%], 99[15%], 71[17%], 43[8%], 15[3%]. Anal. Calcd for $C_{13}H_{16}N_4O$: C, 63.93; H, 6.56; N, 22.95. Found: C, 63.95; H, 6.54; N, 22.93.

4.2.2.3. 3-[2-(Butan-2-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 4. Reagents: Compound **1** (1.0 g, 5.7 mmol), butan-2-one (0.51 ml, 5.7 mmol), ethanol (20 ml). Yield 0.68 g (51.9%). Mp 268–269 °C. IR (cm^{-1}, KBr): ν_{max} 3275(N–H), 1690(C=O), 1570(C=N), 1220(m). λ_{max} (log ε_{max}): 220(3.41), 348(3.62s), 355(3.37). ^1H NMR [300 MHz, CH_3OH-d_4]: 0.86(t, 3H, CH_3), 1.57(q, 2H, CH_2), 1.94(s, 3H, CH_3), 7.00(s, 1H, NH, D_2O, exchangeable). 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.1(C=O), 158.0, 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 26.9(CH_2), 13.9(CH_3), 11.1(CH_3). MS: in *m/z*[rel.%]: 230[M^+, 40%], 202[100%], 103[58%], 29[5%]. Anal. Calcd for $C_{12}H_{14}N_4O$: C, 62.61; H, 6.09; N, 24.35. Found: C, 62.58; H, 6.06; N, 24.30.

4.2.3. General procedure of 3-{2-[1-(6-substituted-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1*H*)-one 5–8

To a ground mixture of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) and unsubstituted or 6-substituted 3-acetylcoumarin (5.7 mmol), was added dry DMF (20 ml) in a 250 ml round-bottomed flask. The reaction mixture was irradiated in a microwave oven at 400 W for 1 min. and the solvent was distilled off. The solid product obtained was filtered, dried and recrystallized from methanol to afford **5–8**.

4.2.3.1. 3-{2-[1-(2-Oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1H)-one, 5. Reagents: Compound **1** (1.0 g, 5.7 mmol), 3-acetylcoumarin (1.07 g, 5.7 mmol), DMF (20 ml), ethanol (20 ml). Yield: 1.6 g (84.0%). Mp 238–241 °C. IR (cm^{-1}, KBr): ν_{max} 3240(N–H), 1740(C=O ester), 1685(C=O), 1606(C=C), 1563(C=N), 1381(C–O). λ_{max} (log ε_{max}): 212(4.58), 327(3.65s), 344(3.68), 365(3.32s). ^1H NMR [300 MHz, CH_3OH-d_4]: 2.07(s, 3H, CH_3), 7.00(s, 1H, NH, D_2O, exchangeable). 7.09–8.27(m, 4H, Ar-H), 7.54(s, 1H, C=CH), 7.42–7.84(m, 4H, Chrom-H), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 159.4(C=O), 158.0(C=O), 157.6, 155.6, 153.0, 142.7, 133.5, 131.7, 129.1, 128.3, 127.9, 125.9, 125.4, 123.5, 123.3, 118.1, 116.1, 115.2, 4.0. MS: in *m/z*[rel.%]: 346[M^+, 35%], 331[53%], 201[13%], 186[82%], 161[100%], 15[3%]. Anal. Calcd for $C_{19}H_{14}N_4O_3$: C, 65.90; H, 4.05; N, 16.18. Found: C, 65.94; H, 4.08; N, 16.21.

4.2.3.2. 3-{2-[1-(6-Bromo-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1*H*)-one, 6. Reagents: Compound **1** (1.0 g, 5.7 mmol), 6-bromo-3-acetylcoumarin (1.52 g, 5.7 mmol), DMF (20 ml), ethanol (20 ml). Yield: 2.08 g (86.0%). Mp 265–266 °C. IR (cm^{-1}, KBr): ν_{max} 3135(N–H), 1740(C=O), 1665(C=O), 1575(C=N), 1288(m), 1130(m). λ_{max} (log ε_{max}): 210(4.10), 325(3.51), 345(3.41), 414(3.32). ^1H NMR [300 MHz, CH_3OH-d_4]: 2.07(s, 3H, CH_3), 7.00(s, 1H, NH, D_2O, exchangeable). 7.09–8.27(m, 4H, Ar-H), 7.31–8.19(m, 3H, Chrom-H), 7.54(s, 1H, C=CH), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 159.4(C=O), 158.0(C=O), 157.6, 155.6, 152.0, 142.7, 134.2, 133.5, 131.7, 130.3, 129.1, 125.9, 124.4, 123.5, 123.3, 119.8, 118.2, 115.2, 4.0. MS: in *m/z*[rel.%]: 425[M^+, 38%], 345[12%], 161[100%], 155[63%], 75[25%]. Anal. Calcd for $C_{19}H_{13}N_4O_3Br$: C, 53.65; H, 3.06; N, 13.18. Found: C, 53.66; H, 3.08; N, 13.21.

4.2.3.3. 3-{2-[1-(6-Chloro-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1*H*)-one, 7. Reagents: Compound **1** (1.0 g, 5.7 mmol), 6-chloro-3-acetylcoumarin (1.27 g, 5.7 mmol), DMF (20 ml), ethanol (20 ml). Yield: 1.97 g (91.0%). Mp 245–246 °C. IR (cm^{-1}, KBr): ν_{max} 3387(N–H), 1740(C=O ester), 1648(C=O), 1612(C=C), 1570(C=N), 1375(C–O ester), 1290(m). λ_{max} (log ε_{max}): 224(4.11), 310(3.71s), 332(3.41), 360(3.97s), 396(4.22). ^1H NMR [300 MHz, CH_3OH-d_4]: 2.07(s, 3H, CH_3), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 7.54(s, 1H, C=CH), 7.36–8.02(m, 3H, Chrom-H), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 159.4(C=O), 158.0(C=O), 157.6, 155.6, 151.1, 142.7, 133.5, 131.7, 131.0, 129.5, 129.1, 126.8, 125.9, 123.6, 123.5, 123.3, 118.0, 115.2, 4.0. MS: in *m/z*[rel.%]: 380.5[M^+, 40%], 345[15%], 160[100%], 15[5%]. Anal. Calcd for $C_{19}H_{13}N_4O_3Cl$: C, 59.92; H, 3.42; N, 14.72. Found: C, 59.90; H, 3.39; N, 14.70.

4.2.3.4. 3-{2-[1-(6-Methyl-2-oxo-2*H*-chromen-3-yl)ethylidene]hydrazinyl}quinoxalin-2(1*H*)-one, 8. Reagents: Compound **1** (1.0 g, 5.7 mmol), 6-methyl-3-acetylcoumarin (1.15 g, 5.7 mmol), DMF (20 ml), ethanol (20 ml). Yield: 1.5 g (73.2%). Mp 183–185 °C. IR (cm^{-1}, KBr): ν_{max} 3118(N–H), 1742(C=O), 1665(C=O), 1571(C=N), 1273(m). λ_{max} ($\log \varepsilon_{max}$): 210(3.11), 320(3.67), 342(3.17), 350(3.84), 360(3.51), 408(3.57). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 2.07(s, 3H, CH_3), 2.34(s, 3H, CH_3), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 7H, 2 × Ar-H), 7.54(s, 1H, C=CH), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 159.4(C=O), 158.0(C=O), 157.6, 155.6, 150.0, 142.7, 135.1, 133.5, 132.0, 131.7, 129.1, 127.0, 125.9, 123.5, 123.3, 122.1, 116.9, 115.2, 21.7, 5.0. MS: in *m/z*[rel.%]: 360[M^+, 35%], 346[20%], 161[100%], 158[60%], 72[18%], 15[7%]. Anal. Calcd for $C_{20}H_{16}N_4O_3$: C, 66.67; H, 4.44; N, 15.56. Found: C, 66.68; H, 4.47; N, 15.59.

4.2.4. 3-(2-Cyclopentylidenehydrazinyl)quinoxalin-2(1*H*)-one, 9

To a solution of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) in cyclopentanone (0.50 ml, 5.7 mmol), was added 50% aqueous ethanol (20 ml) in an open beaker over heated alumina. The mixture was irradiated in microwave oven at an emitted power of 400 W for 3 min to get a clear solution. The clear solution formed was left to stand at room temperature to crystallize. The solid formed was recrystallized from ethanol to afford **9** 1.08 g (78.3%). Mp 249–250 °C. IR (cm^{-1}, KBr): ν_{max} 3120(N–H), 1675(C=O). λ_{max} ($\log \varepsilon_{max}$): 220(3.11), 344(3.89), 350(3.35s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 1.30(quintet, 4H, 2 × CH_2), 2.35(t, 4H, 2 × CH_2), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O, exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 187.7(C=N Cp), 158.0(C=O), 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 35.7, 29.7, 24.8, 24.8. MS: in *m/z*[rel.%]: 242[M^+, 75%], 161[100%], 118[68%], 78[10%]. Anal. Calcd for $C_{13}H_{14}N_4O$: C, 64.46; H, 5.79; N, 23.14. Found: C, 64.41; H, 5.74; N, 23.10.

4.2.5. General procedure of 5-substituted-1*H*-indole-2,3-dione 3-[(3-oxo-3,4-dihydroquinoxalin-2-yl)hydrazone], 10–13

To a ground mixture of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) and isatin or 5-substituted-isatin (5.7 mmol), 1:5 of DMF/ethanol (30 mL) was added dropwise in a 250 ml round-bottomed flask. The reaction mixture was irradiated in microwave oven at an emitted power of 400 W for 2 min. Then, it was poured into crushed ice and the product was filtered and recrystallized from DMF/water to give **10–13**.

4.2.5.1. 3-[2-(2-Oxoindolin-3-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 10. Reagents: Compound **1** (1.0 g, 5.7 mmol), isatin (0.8 g, 5.7 mmol), 1:5 of DMF/ethanol (30 ml). Yield: 1.6 g (90.8%). Mp 323–324 °C. IR (cm^{-1}, KBr): ν_{max} 3138(N–H), 1705(C=O), 1665(C=O), 1618(C=C), 1533(C=N), 1338, 1290, 1180, 1132. λ_{max} ($\log \varepsilon_{max}$): 228(4.22), 272(4.13), 348(3.18s), 370(3.49s), 424(3.56). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 8H, 2 × Ar-H), 8.00(s-br, 2H, 2 × NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 168.5(C=O), 158.0(C=O), 157.9, 142.7, 141.2, 134.5, 131.7, 131.2, 129.4, 129.1, 125.9, 124.4, 123.5, 119.4, 117.7, 115.2. MS: in *m/z*[rel.%]: 305[M^+, 68%], 161[100%], 145[35%], 91[15%]. Anal. Calcd for $C_{16}H_{11}N_5O_2$: C, 62.95; H, 3.61; N, 22.95. Found: C, 62.95; H, 3.64; N, 22.90.

4.2.5.2. 3-[2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 11. Reagents: Compound **1** (1.0 g, 5.7 mmol), 5-bromoisatin (1.29 g, 5.7 mmol), 1:5 of DMF/ethanol (30 ml). Yield: 2.01 g (91.7%). Mp 328–330 °C. IR (cm^{-1}, KBr): ν_{max} 3140(N–H), 1690(C=O), 1665(C=O), 1531(C=N), 1344, 1298, 1185, 1137. λ_{max} ($\log \varepsilon_{max}$): 225(4.10), 270(4.01), 345(3.21), 420(3.71). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 7.56–8.10(m, 3H, In-H), 8.00(s-br, 2H, 2 × NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 168.5(C=O), 158.0(C=O), 157.9, 142.7, 140.2, 136.9, 134.5, 132.9, 131.7, 129.1, 125.9, 123.5, 119.9, 118.8, 118.0, 115.2. MS: in *m/z*[rel.%]: 384[M^+, 55%], 304[65%], 160[97%], 90[13%]. Anal. Calcd for $C_{16}H_{10}N_5O_2Br$: C, 50.00; H, 2.60; N, 18.23. Found: C, 50.04; H, 2.65; N, 18.23.

4.2.5.3. 3-[2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 12. Reagents: Compound **1** (1.0 g, 5.7 mmol), 5-chloroisatin (1.03 g, 5.7 mmol), 1:5 of DMF/ethanol (30 ml). Yield: 1.70 g (93.4%). Mp 326 °C (dec.). IR (cm^{-1}, KBr): ν_{max} 3145(N–H), 1705(C=O), 1670(C=O), 1533(C=N), 1349, 1188, 1139 cm^{-1}. λ_{max} ($\log \varepsilon_{max}$): 210(3.74), 265(3.91), 340(3.46), 421(4.01). ^{1}H NMR [300 MHz, DMSO-d_6]: 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 7.50–7.89(m, 3H, In-H), 8.00(s-br, 2H, 2 × NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 168.5(C=O), 158.0(C=O), 157.6, 142.7, 139.3, 134.5, 131.7, 131.3, 130.0, 129.4, 129.1, 125.9, 125.2, 123.5, 119.2, 115.2. MS: in *m/z*[rel.%]: 339.5[M^+, 63%], 304[60%], 161[100%], 125.5[18%]. Anal. Calcd for $C_{16}H_{10}N_5O_2Cl$: C, 56.55; H, 2.95; N, 20.62. Found: C, 56.51; H, 2.93; N, 20.58.

4.2.5.4. 3-[2-(5-Methyl-2-oxoindolin-3-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 13. Reagents: Compound **1** (1.0 g, 5.7 mmol), 5-methylisatin (0.92 g, 5.7 mmol), 1:5 of DMF/ethanol (30 ml). Yield: 1.61 g (88.4%). Mp 257–259 °C. IR (cm^{-1}, KBr): ν_{max} 3120(N–H), 1690(C=O), 1665(C=O), 1570(C=N), 1322, 1175. λ_{max} ($\log \varepsilon_{max}$): 223(3.11), 267(3.78), 344(3.88), 425(4.12). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 2.34(s, 3H, CH_3), 6.66–8.21(m, 3H, In-H), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s-br, 2H, 2 × NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 168.5(C=O), 158.0(C=O), 157.6, 142.7, 138.2, 134.5, 134.1, 131.7, 131.5, 129.6, 129.1, 125.9, 123.5, 121.6, 117.6, 115.2, 21.3(CH_3). MS: in *m/z*[rel.%]: 319[M^+, 73%], 304[51%], 161[100%], 145[29%], 90[10%]. Anal. Calcd for $C_{17}H_{13}N_5O_2$: C, 63.95; H, 4.08; 21.94. Found: C, 63.99; H, 4.04; N, 21.90.

4.2.5.5. 3-[2-(Anthracen-9(10*H*)-ylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 14. To a ground mixture of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) and anthrone (1.1 g, 5.7 mmol), was added 1:4 of DMF/ethanol (20 ml). The reaction mixture was irradiated in microwave oven over heated alumina at an emitted power of 400 W for 75 s to give a clear solution. The crude product obtained was recrystallized from ethanol to afford **14** 2.0 g (99.5%). Mp 229–231 °C. IR (cm^{-1}, KBr): ν_{max} 3411(N–H), 1705(C=O), 1575(C=N). λ_{max} ($\log \varepsilon_{max}$): 212(3.73), 276(3.50), 352(3.38s), 376(3.14s), 398(3.11s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 3.81(s, 2H, CH_2), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 7.20–7.85(m, 8H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.0(C=O), 157.6, 142.7, 139.4, 139.4, 133.0, 133.0, 131.7, 131.5, 131.5, 129.7, 129.7, 129.1, 128.3, 128.3, 127.5, 126.3, 126.3, 125.9, 123.5, 115.2, 32.5(CH_2). MS: in *m/z*[rel.%]: 351[M^+, 85%], 174[100%], 76[23%]. Anal. Calcd for $C_{22}H_{15}N_4O$: C, 75.21; H, 4.27; N, 15.95. Found: C, 75.17; H, 4.22; N, 15.91.

4.2.5.6. 3-{2-(1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ylidene)hydrazinyl}quinoxalin-2(1*H*)-one, 15. To a solution of Camphor (0.9 g, 5.7 mmol) in ethanol (15 ml) was added homogenous mixture of 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) and 1:5 of DMF/ethanol (30 ml) in an open beaker. The resulting mixture was irradiated in microwave oven at 400 W for 1 min. and allowed to cool at room temperature. The crude product obtained was recrystallized from ethanol to afford **15** 1.0 g (56.0%). Mp 331–333 °C. IR (cm^{-1}, KBr): ν_{max} 3272(N–H), 1685(C=O),

1588(C=N). λ_{max} (log ε_{max}): 216(4.31), 328(3.59), 350(3.56s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 0.99(s, 6H, 2 × CH_3), 1.10(s, 3H, CH_3), 1.32(m, 2H, CH_2), 1.50(m, 1H, CH), 1.60(m, 2H, CH_2), 2.61–2.86(m, 2H, CH_2), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 166.5(C=O), 158.0, 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 51.2, 47.0, 43.7, 35.1, 31.8, 27.5, 19.6, 19.6, 11.2. MS: in *m/z*[rel.%]: 310[M^+, 89%], 160[95%], 150[100%], 72[15%], 15[2%]. Anal. Calcd for $C_{18}H_{22}N_4O$: C, 69.68; H, 7.10; N, 18.06. Found: C, 69.71; H, 7.14; N, 18.09.

4.2.6. General procedure of 3-[2-(2-substitutedcyclohexylidene) hydrazinyl]quinoxalin-2(1*H*)-one 16–19

Pure and dry 3-hydrazinoquinoxalin-2(1*H*)-one, **1** (1.0 g, 5.7 mmol) was added in small portion to cyclohexanone or 2-substitutedcyclohexanone (5.7 mmol) with continuous stirring, followed by the addition of ethanol (30 ml). The reaction mixture was irradiated in microwave oven at an emitted power of 400 W for 2 min over bentonite support. The resulting solution was cooled over crushed ice to give crude product. The crude product obtained was recrystallized from ethanol to afford **16–19.**

4.2.6.1. 3-(2-Cyclohexylidenehydrazinyl)quinoxalin-2(1*H*)-one, 16. Reagents: Compound **1** (1.0 g, 5.7 mmol), cyclohexanone (0.60 ml, 5.7 mmol), ethanol (30 ml). Yield: 0.90 g (61.4%). Mp 201–203 °C. IR (cm^{-1}, KBr): ν_{max} 3225(N–H), 1667(C=O), 1575(C=N). λ_{max} (log ε_{max}): 220(3.81), 233(4.10), 345(3.52s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 1.60–1.67(m, 6H, 3 × CH_2), 2.34(t, 4H, 2 × CH_2), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 161.7(C=O), 158.0, 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 34.1, 28.1, 27.1, 27.1, 25.5. MS: in *m/z*[rel.%]: 257[M^+, 37%], 161[92%], 111[100%], 70[28%]. Anal. Calcd for $C_{14}H_{16}N_4O$: C, 65.63; H, 6.25; N, 21.88. Found: C, 65.60; H, 6.22; N, 21.84.

4.2.6.2. 3-[2-(2-Bromocyclohexylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 17. Reagents: Compound **1** (1.0 g, 5.7 mmol), 2-bromocyclohexanone (0.84 ml, 5.7 mmol), ethanol (30 ml). Yield: 1.42 g (73.9%). Mp 176–178 °C. IR (cm^{-1}, KBr): ν_{max} 3235(N–H), 1670(C=O), 1577(C=N). λ_{max} (log ε_{max}): 225(3.62), 235(3.96), 346(3.48s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 1.18–1.34(m, 6H, 3 × CH_2), 1.70–1.90(m, 2H, CH_2), 4.34(t, 1H, CH–Br), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.0(C=O), 157.6, 155.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 41.6(C–Br), 38.2, 26.4, 24.3, 23.2. MS: in *m/z*[rel.%]: 336[M^+, 49%], 256[40%], 190[100%], 175[70%]. Anal. Calcd for $C_{14}H_{15}N_4OBr$: C, 50.15; H, 4.48; N, 16.72. Found: C, 50.18; H, 4.50; N, 16.76.

4.2.6.3. 3-[2-(2-Chlorocyclohexylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 18. Reagents: Compound **1** (1.0 g, 5.7 mmol), 2-chlorocyclohexanone (0.66 ml, 5.7 mmol), ethanol (30 ml). Yield: 1.47 g (88.3%). Mp 162–163 °C. IR (cm^{-1}, KBr): ν_{max} 3241(N–H), 1685(C=O), 1577(C=N). λ_{max} (log ε_{max}): 225(3.41), 238(3.18), 340(3.73s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 1.17–1.32(m, 6H, 3 × CH_2), 1.40–1.70(m, 2H, CH_2), 3.50(t, 1H, CH–Cl), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.0(C=O), 157.6, 155.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 53.7(C-Cl), 30.4, 26.6, 23.0, 21.9. MS: in *m/z*[rel.%]: 291.5[M^+, 53%], 256[39%], 145.5[100%], 130.5[73%]. Anal. Calcd for $C_{14}H_{15}N_4OCl$: C, 57.83; H, 5.16; N, 19.27. Found: C, 57.88; H, 5.20; N, 19.31.

4.2.6.4. 3-[2-(2-Methylcyclohexylidene)hydrazinyl]quinoxalin-2(1*H*)-one, 19. Reagents: Compound **1** (1.0 g, 5.7 mmol), 2-methylcyclohexanone (0.69 ml, 5.7 mmol), ethanol (30 ml). Yield: 0.85 g (55.2%). Mp 282–284 °C. IR (cm^{-1}, KBr): ν_{max} 3205(N–H), 1663(C=O), 1570(C=N). λ_{max} (log ε_{max}): 230(3.73), 238(3.41), 350(3.73s). ^{1}H NMR [300 MHz, CH_3OH-d_4]: 0.91(d, 3H, CH_3), 1.18–1.40(m, 8H, 4 × CH_2), 1.36(m, 1H, CH), 7.00(s, 1H, NH, D_2O, exchangeable), 7.09–8.27(m, 4H, Ar-H), 8.00(s, 1H, NH, D_2O exchangeable). ^{13}C NMR [300 MHz, CH_3OH-d_4]: 158.5(C=O), 158.0, 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 33.1, 31.8, 27.3, 24.1, 20.2, 17.6(CH_3). MS: in *m/z*(rel.%): 271[M^+, 66%], 256[36%], 125[100%], 84[15%], 15[7%]. Anal. Calcd for $C_{15}H_{18}N_4O$: C, 66.67; H, 6.67; N, 20.74. Found: C, 66.69; H, 6.68; N, 20.79.

4.3. Antibacterial activity assays

Most of the organisms used were standard bacteria of National Collection for Industrial Bacteria (NCIB) while few others were Locally Isolated Organisms (LIO). The organisms were *B. cereus* (NCIB 6349), *B. stearothermophilus* (NCIB 8222), *B. subtilis* (NCIB 3610), *B. anthracis* (LIO), *Bacillus polymyxa* (LIO), *Corynebacterium pyogenes* (LIO), *Streptococcus faecalis* (NCIB775), *S. aureus* (NCIB 8588), *Clostridium sporogenes* (LIO), *E. coli* (NCIB 86), *P. fluorescence* (NCIB 3756), *K. pneumonia* (NCIB 418), *S. dysenteriae* (LIO), *P. aeruginosa* (NCIB 950) and *C. albican* (LIO).

4.3.1. Antibacterial sensitivity testing of compounds, 1–19

All the synthesized compounds **(1–19)** and Streptomycin were screened for antibacterial activity on nine gram positive and five gram negative bacterial strains using agar well diffusion method.[49] The medium employed was diagnostic sensitivity test agar (Biotech Ltd). With the aid of a sterile 1 ml pipette, about 0.2 ml of the broth culture of test organism was added to 18 ml sterile molten diagnostic sensitivity test agar (Biotech Ltd) which had already cooled down to 45 °C. This was well mixed and poured into previously sterilized petri dishes, which had been properly labeled according to the test organisms. The medium was then allowed to set. With the aid of a sterile cork borer, the required numbers of holes were bored into the medium. The wells were made of about 5 mm to the edge of the plate. The wells were then filled up aseptically with the solution of the compound in DMSO using Pasteur pipettes. Streptomycin was used as the standard antibacterial agent at a concentration of 1000 μg/ml. The plates were allowed to stand for about 1 h on the bench for proper diffusion of the antibacterial agents into the medium and then incubated uprightly at 37 °C for 24 h. Care was taken not to stockpile the plates. Clear zones of inhibition in millimetres indicated the relative susceptibility of the bacteria to the compounds **(1–19)** and Streptomycin standard.

4.3.2. Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was done using the method of Russell and Furr.[49] Based on the level of resistance of some organisms and large zones of inhibition experienced in others, Minimum Inhibitory Concentration (MIC) was selectively done for five gram positive and five gram negative bacterial strains. Different concentrations (7.8 μg/ml and 1000.0 μg/ml) of the compounds and standard were prepared using a twofold dilution which was prepared in a sterile plate with the aid of sterile pipette and then mixed with 18 ml of molten nutrient agar. This was then allowed to set. The surface of the nutrient agar plate was allowed to dry before streaking with overnight broth cultures of the bacterial strains. The plates were then labeled accordingly and incubated at 37 °C for up to 72 h. They were subsequently examined for the presence or absence of growth. The lowest concentration

preventing the growth of bacteria was taken as the Minimum Inhibitory Concentration of the compounds. This procedure was likewise repeated for the Streptomycin (standard).

4.3.3. Determination of antifungal activity

The antifungal activities of the synthesized compounds were determined by placing one disc (3 mm diameter) of a 3 day old culture of the *C. albicans* in each of the triplicates Petri dishes (11 cm diameter with 160 ml Potato Dextrose Agar (PDA) medium and 3 ml of the different synthesized compounds (**1**–**19**) The control experiments were set up with 3 ml of Nystatin (500,000 IU) using same media. This was done in triplicate plates and was incubated at room temperatures (28 ± 2 °C) for three days. Daily measurements of the mycelia extension of the cultures were determined by measuring culture along diameters and comparing with mycelial growth of the control.[50] The difference in their diameters reflects the extent of inhibition by the synthesized compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.064.

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