J. Chem. Sci. Vol. 125, No. 1, January 2013, pp. 129–139.

Synthesis of 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide-based azetidinone derivatives as potent antibacterial and antifungal agents

SHIV KUMAR[a,*], NITIN KUMAR[b], SUSHMA DRABU[c] and MD. AKRAM MINHAJ[a]

[a]Division of Pharmaceutical Chemistry, Department of Pharmacy, IEC College of Engineering and Technology, Knowledge Park-I, Greater Noida 201 308, India

[b]Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut 250 005, India

[c]Maharaja Surajmal Institute of Pharmacy, Janak Puri, New Delhi 110 058, India

e-mail: shiv_hamdard@yahoo.co.in; shiv_hamdard@rediffmail.com

MS received 17 January 2012; revised 15 June 2012; accepted 28 June 2012

Abstract. Twelve compounds belonging to series *N*-[3-chloro-2-oxo-4-(substituted)phenylazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide (**5a–l**) were synthesized. These compounds were evaluated for their *in vitro* antibacterial against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and antifungal activity against *C. albicans*, *A. niger* and *A. flavus* by cup-plate method. Structures of all the newly synthesized compounds were confirmed by their spectral data interpretation. Compound **5g** having *p*-dimethylaminophenyl group on 4-position of azetidinone ring attached to N-atom of acetamido group on 1-position of 3-methyl-1*H*-quinoxaline-2-one, was found to be active against all the bacterial and fungal strains under investigation.

Keywords. Quinoxaline; azetidinone; antibacterial; antifungal.

1. Introduction

Nitrogen containing heterocyclic compounds are indispensable structural units for both the chemists and biochemists. Among the various classes of benzene fused six-membered nitrogen containing heterocyclic compounds, quinoxaline derivatives form an important class of pharmacologically active compounds. Quinoxaline ring is a part of various antibiotics such as hinomycin, levomycin, and actinoleutin that are known to inhibit growth of Gram positive bacteria and are active against various transplantable tumours.[1–7] Quinoxaline derivatives have also been found to be associated with a wide variety of biological activities including antifungal,[8–10] antibacterial,[10–14] antitubercular,[8,9,15–18] antiinflammatory agents.[19] Further, hydrazinoquinoxalines and their cyclic analogues have been reported as antimicrobial agents.[20] Similarly, four-member nitrogen containing heterocyclic ring azetidinone has also been associated with compounds possessing various pharmacological activities viz. antibacterial,[21,23,24] antiinflammatory,[21] antifungal,[22–24] antitubercular[22] etc.

Azetidin-2-one (β-lactam) ring is present in several widely used families of antibiotics such as penicillins, cephalosporins, carbapenems, and monocyclic β-lactams (for example aztreonam, a potent inhibitor of cephalosporinase). Recent discoveries have proved that β-lactams can serve as mechanism based inhibitors of serine protease.[25,26] Subsequently 2-azetidinones were highlighted as a potent mechanism based inhibitor of several enzymes like human tryptase, chymase, thrombin, leukocyte elastase, human cytomegalovirus protease.[27]

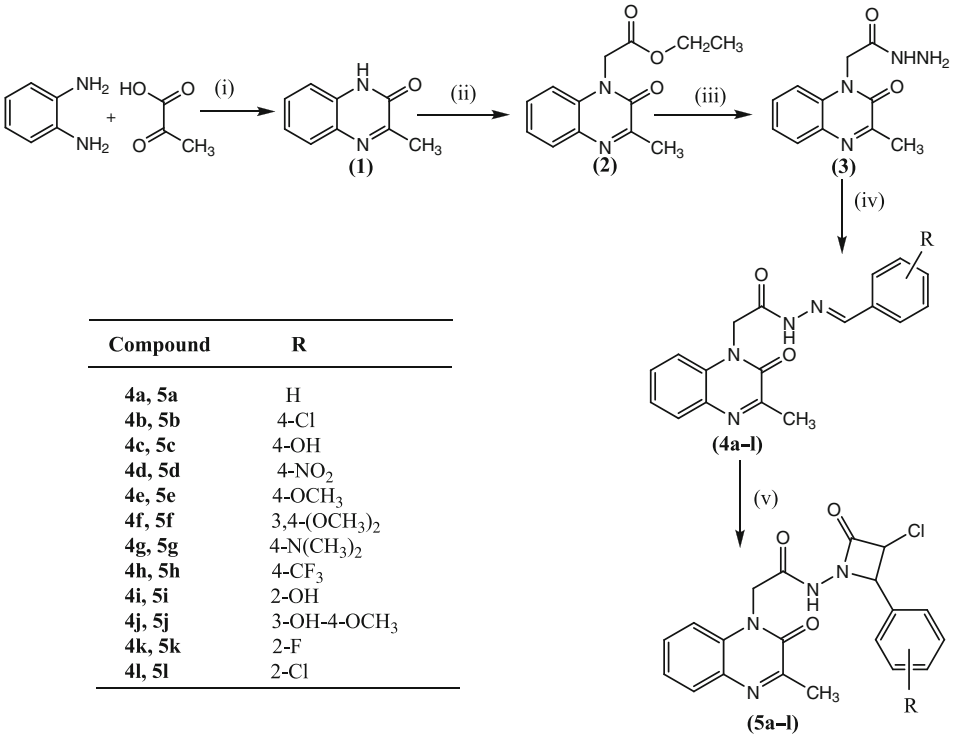
Keeping the above facts in view, it was thought worthwhile to design the synthesis of compound series viz. *N*-[3-chloro-2-oxo-4-(substituted)phenylazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide **(5a–l)** wherein azetidinone ring has been attached to quinoxaline at 1- position with an intervening acetamido amino linkage. All the synthesized compounds have been screened for *in vitro* antibacterial activity against Gram positive bacteria *Staphylococcus aureus* (ATCC2913), Gram negative bacteria *Klebsiella pneumoniae* (ATCC700603), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC25922) and antifungal activity against *Candida albicans* (MTCC3017), *Aspergillus niger* (MTCC281) and

*For correspondence

Table 1. Zone of inhibition (mm) of different compounds.

	E. coli		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*C. albicans*		*A. niger*		*A. flavus*	
Compound	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL
5a	10	18	11	19	10	18	12	21	11	19	10	17	11	20
5b	12	21	13	22	13	22	14	23	10	17	10	18	11	19
5c	10	18	13	22	14	25	13	21	12	21	09	15	10	18
5d	12	22	12	23	13	24	13	22	11	19	11	20	11	19
5e	09	15	10	17	10	18	10	17	09	16	10	17	08	14
5f	11	19	10	18	11	20	11	19	10	17	11	19	11	20
5g	12	21	12	22	13	23	13	22	13	23	12	21	12	22
5h	13	23	14	25	14	24	14	25	13	23	11	19	13	23
5i	09	16	09	15	08	14	09	15	10	18	10	17	08	13
5j	12	21	12	22	11	18	12	20	12	21	13	22	11	19
5k	09	15	09	16	09	16	10	17	10	16	08	14	07	12
5l	10	17	10	18	09	15	09	16	08	13	09	15	08	13
Standard	27		28		27		27		26		26		25	
Control	02		02		02		02		02		03		02	

Standard drugs
For antibacterial activity: Ciprofloxacin 30 μg/mL
For antifungal activity: Voriconazole 30 μg/mL
Control: DMSO



Compound	R
4a, 5a	H
4b, 5b	4-Cl
4c, 5c	4-OH
4d, 5d	4-NO_2
4e, 5e	4-OCH_3
4f, 5f	3,4-$(OCH_3)_2$
4g, 5g	4-$N(CH_3)_2$
4h, 5h	4-CF_3
4i, 5i	2-OH
4j, 5j	3-OH-4-OCH_3
4k, 5k	2-F
4l, 5l	2-Cl

Reagents and conditions: (i) 20% HCl, reflux, (ii) $ClCH_2COOCH_2CH_3$, K_2CO_3, acetone, reflux, (iii) $NH_2NH_2.H_2O$, EtOH, reflux, (iv) R-C_6H_4-CHO, MeOH, reflux, (v)Et_3N, $ClCH_2COCl$, dry benzene, 0°–5°C, stirring

Figure 1. Reaction scheme.

Aspergillus flavus (MTCC222) by using cup-plate method (figure 1, table 1).

2. Experimental

2.1 *General*

Melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. All the Fourier Transform-Infra Red (FT-IR) spectra were recorded in KBr pellets on Shimadzu-8400S spectrometer. The ^{1}H-NMR spectra were taken on Bruker-Spectrospin DCX (300 MHz) NMR spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. The ESI mass spectra of a few representative compounds were recorded on a MICRO MASS QUATTRO II triple quadrupole mass spectrometer. Elemental analysis was performed on Flash EA 1112 Thermo Electron Corporation CHNS analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) on Merck Silica Gel 60_{F254} pre-coated sheets. Iodine chamber and UV lamp were used for the visualization of TLC spots.

2.2 *General procedure for the synthesis of titled compounds*

2.2a *3-Methyl-1H-quinoxaline-2-one* ***(1)***: Pyruvic acid (0.1 mol) and *o*-phenylenediamine (0.1 mol), in 20% HCl (100 mL), were mixed properly and heated at 45°C with continuous stirring for about 5 h. Product was filtered, dried and washed with water, dried and then purified by dissolving in 5% w/v NaOH (75 mL). Then, liquid was filtered and cooled to temperature below 5°C and acidified with glacial acetic acid to pH 6. Buff coloured crystals appeared and then recrystallized with dimethylformamide (DMF). Pale yellow crystals, yield 60%, $R_{f\ (\text{Ethyl acetate : Hexane} = 7:3)}$ 0.73, m.p. 230°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 7.62–7.65 (d, 1H, quinoxaline ring protons, J = 8.1 Hz), 7.39–7.44 (t, 1H, quinoxaline ring protons, J = 5.4–9.6 Hz), 7.18–7.25 (m, 2H, quinoxaline ring protons, J = 2.7–7.5 Hz), 2.36 (s, 3H, CH_3), 9.1 (s, 1H, NHCO); IR (KBr, cm^{-1}): 762 (*ortho*-disubstituted aromatic ring), 1390 (C–N), 1500 (aromatic C=C), 1544 (-NH), 1688 (>C=O), 3314 (-NH).

2.2b *Ethyl-[3-methyl-2-oxo-quinoxaline-1-yl]acetate* ***(2)***: Compound (**1**) (0.0642 mol), ethylchloroacetate

(0.077 mol) and potassium carbonate (0.078 mol) were taken in round bottom flask and refluxed in acetone (100 mL) for 6 h. After completion of reaction, acetone was removed by reduced pressure. The residue was added to ice cold water (300 mL), acidified with acetic acid, filtered, washed with cold water, dried and finally recrystallized with ethyl acetate. Pale yellow crystals, yield 45%, $R_{f\ (\text{Ethyl acetate : Hexane} = 7:3)}$ 0.64, m.p. 112°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 7.73–7.76 (d, 1H, quinoxaline ring protons, J = 8.1 Hz), 7.53–7.55 (t, 1H, quinoxaline ring protons, J = 3.9–4.2 Hz), 7.43–7.46 (d, 1H, quinoxaline ring protons, J = 3 Hz), 7.31–7.36 (t, 1H, quinoxaline ring protons, J = 6.6–8.4 Hz), 4.11–4.18 (q, 2H, CH_2OCO), 3.38 (s, 2H, CH_2CO), 2.39 (s, 3H, CH_3), 1.14–1.19 (t, 3H, CH_3); IR (KBr, cm^{-1}): 763 (*ortho*-disubstituted aromatic ring), 962 (aromatic =C–H, bend), 1199 (=C–N), 1285 (C–O, coupled with H–C–H), 1380 (C–N), 1645 (CH=N), 1750 (>C=O), 2745 (C–C).

2.2c *3-Methyl-2-oxo-quinoxaline-1(2H)-acetohydrazide* ***(3)***: Compound 2 (0.02 mol) and hydrazine hydrate (0.03 mol) were dissolved in ethanol (50 mL) and refluxed for 6 h. Reaction mixture was kept in deep freezer for overnight. Product was filtered, dried and finally recrystallized with DMF. Biege coloured solid, yield 50%, $R_{f\ (\text{Toluene : Ethyl acetate ; Formic acid, TEF} = 5:4:1)}$ 0.9, m.p. 200°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 7.72–7.75 (d, 1H, quinoxaline ring protons, J = 7.8 Hz), 7.49–7.54 (t, 1H, quinoxaline ring protons, J = 6.3–9 Hz), 7.26–7.35 (q, 2H, quinoxaline ring protons, J = 7.5–10.2 Hz), 4.84 (s, 2H, $NHNH_2$), 4.28 (s, 2H, CH_2CO), 2.43 (s, 3H, CH_3); IR (KBr, cm^{-1}): 760 (*ortho*-disubstituted aromatic ring), 963 (aromatic =C–H, bend), 1198 (=C–N), 1385 (C–N), 1569 (-NH, bend), 1645 (CH=N), 1749 (>C=O), 2320 (N–N), 3245 (-NH. str.).

2.2d *2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N′-[(substituted)phenylmethylidene]aceto hydrazide* ***(4a–l)***: Equimolar quantities of compound **3** (0.0044 mol) and aromatic aldehydes (0.0045 mol) were dissolved in methanol (25 mL) and refluxed for 6 h. A solid appears with vigorous bumping in each case. Solid was filtered, washed with cold methanol, dried and recrystallized with DMF.

2.2e *N′-benzylidene-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4a)***: Light yellow solid, yield 54%, $R_{f\ (\text{Benzene : Acetone} = 8:2)}$ 0.85, m.p. 234°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.06 (s, 1H, NHCO), 7.67–7.77 (m, 4H, quinoxaline ring protons, J = 3–7.5 Hz), 7.41–7.53 (m, 5H, Ar–H, J = 2.7–8.1 Hz), 5.42 (s, 2H, CH_2), 5.03 (s, 1H, CH=N), 2.54 (s, 3H, CH_3); IR (KBr, cm^{-1}): 723 (*mono*-substituted aromatic ring), 769 (*ortho*-disubstituted aromatic ring), 854 (aromatic C=C), 964 (aromatic =C–H, bend), 1199 (=C–N) 1388 (C–N), 1542 (-NH, bend), 1650 (CH=N), 1729 (>C=O), 2345 (N–N=), 3244 (-NH).

2.2f *N′-(4-chlorobenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4b)***: Light yellow solid, yield 48%, $R_{f\ (\text{Benzene : Acetone} = 8:2)}$ 0.72, m.p. 230°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.03–8.06 (q, 4H, A_2B_2 pattern, Ar–H, J = 3.3–3.6 Hz), 8.18 (s, 1H, CONH), 7.57–7.76 (m, 4H, quinoxaline ring protons, J = 2.7–8.1 Hz), 5.42 (s, 2H, CH_2), 5.03 (s, 1H, CH=N), 2.35 (s, 3H, CH_3); IR (KBr, cm^{-1}): 765 (*ortho*-disubstituted aromatic ring), 823 (*para*-disubstituted aromatic ring), 865 (aromatic C=C), 965 (aromatic =C–H, bend), 1129 (aryl C–Cl), 1203 (=C–N), 1385 (C–N), 1540 (-NH, bend), 1650 (CH=N), 1740 (>C=O), 2342 (N–N=), 3245 (-NH).

2.2g *N′-(4-hydroxybenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4c)***: Greenish yellow solid, yield 45%, $R_{f\ (\text{Benzene : Acetone} = 8:2)}$ 0.76, m.p. 254°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.02–8.06 (q, 4H, A_2B_2 pattern, Ar–H, J = 3–3.6 Hz), 8.15 (s, 1H, CONH), 7.75–7.87 (m, 4H, quinoxaline ring protons, 2.1–8.4 Hz), 5.32 (s, 2H, CH_2), 5.13 (s, 1H, CH=N), 2.48 (s, 1H, OH), 2.35 (s, 3H, CH_3); IR (KBr, cm^{-1}): 764 (*ortho*-disubstituted aromatic ring), 825 (*para*-disubstituted aromatic ring), 863 (aromatic C=C), 960 (aromatic =C–H, bend), 1198 (=C–N), 1288 (C–O, coupled with H–C–H), 1382 (C–N), 1540 (-NH, bend), 1650 (CH=N), 1745 (>C=O), 2344 (N–N=), 3250 (-NH), 3675 (phenolic -OH).

2.2h *2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N′-(4-nitrobenzylidene)acetohydrazide* ***(4d)***: Grey solid, yield 52%, $R_{f\ (\text{Benzene : Acetone} = 8:2)}$ 0.70, m.p. 220°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.03–06 (q, 4H, A_2B_2 pattern, Ar–H, J = 3–3.3 Hz), 8.15 (s, 1H, CONH), 7.77–7.87 (m, 4H, quinoxaline ring protons, J = 2.4–7.5 Hz), 5.52 (s, 2H, CH_2), 5.03 (s, 1H, CH=N), 2.54 (s, 3H, CH_3); IR (KBr, cm^{-1}): 723 (*ortho*-disubstituted aromatic ring), 829 (*para*-disubstituted aromatic ring), 864 (aromatic C=C), 969 (aromatic =C–H, bend), 1199 (=C–N), 1369 (aromatic –NO_2 *sym.*, str.), 1385 (C–N), 1542 (-NH, bend),

1551 (aromatic $-NO_2$, *asym.*, str.) 1648 (CH=N), 1748 (>C=O), 2341 (N–N=), 3247 (-NH).

2.2i *N′-(4-methoxybenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4e)***: Light yellow solid, yield 43%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.80, m.p. 218°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.02–8.05 (d, 4H, Ar–H, J = 9.6 Hz), 8.14 (s, 1H, CONH), 7.57–7.67 (m, 4H, quinoxaline ring protons, J = 2.4–7.5 Hz), 5.52 (s, 2H, CH_2), 5.02 (s, 1H, CH=N), 3.36 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3); IR (KBr, cm^{-1}): 769 (*ortho*-disubstituted aromatic ring), 825 (*para*-disubstituted aromatic ring), 865 (aromatic C=C), 964 (aromatic =C–H, bend), 1040 (C–O–C, *sym.* str.), 1129 (aromatic C–O), 1198 (=C–N), 1240 (C–O–C, *asym.* str.), 1388 (C–N), 1540 (-NH, bend), 1648 (CH=N), 1745 (>C=O), 2344 (N–N=), 3250 (-NH).

2.2j *N′-(3,4-dimethoxybenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4f)***: Light yellow solid, yield 40%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.72, m.p. 208°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.11 (s, 1H, CONH), 7.81–7.99 (m, 4H, quinoxaline ring protons, J = 2.4–7.5 Hz), 7.77–7.79 (d, 1H, Ar–H, J = 6.3 Hz), 7.67–7.69 (d, 1H, Ar–H, J = 6.6 Hz), 7.62 (s, 1H, Ar–H), 5.33 (s, 2H, CH_2), 5.02 (s, 1H, CH=N), 3.62 (s, 6H, OCH_3), 2.45 (s, 3H, CH_3); IR (KBr, cm^{-1}): 768 (*ortho*-disubstituted aromatic ring), 830 (*para*-disubstituted aromatic ring), 863 (aromatic C=C), 965 (aromatic =C–H, bend), 1042 (C–O–C, *sym.* str.), 1132 (aromatic C–O), 1199 (=C–N), 1245 (C–O–C, *asym.* str.), 1385 (C–N), 1545 (-NH, bend), 1650 (CH=N), 1745 (>C=O), 2344 (N–N=), 3245 (-NH).

2.2k *N′-(4-(dimethylamino)benzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4g)***: Yellow solid, yield 54%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.86, m.p. 228°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 7.03–7.08 (q, 4H, A_2B_2 pattern, Ar–H, J = 3–5.7 Hz), 7.81 (s, 1H, CONH), 7.55–7.67 (m, 4H, quinoxaline ring protons, J = 2.1–8.1 Hz), 5.22 (s, 2H, CH_2), 5.01 (s, 1H, CH=N), 2.35 (s, 3H, CH_3), 1.83 (s, 6H, N–CH_3); IR (KBr, cm^{-1}): 769 (*ortho*-disubstituted aromatic ring), 830 (*para*-disubstituted aromatic ring), 865 (aromatic C=C), 968 (aromatic =C–H, bend), 1199 (=C–N), 1384 (C–N), 1550 (-NH, bend), 1648 (CH=N), 1750 (>C=O), 2344 (N–N=), 3124 (-CH_3), 3245 (-NH).

2.2l *2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N′-(4-(trifluoromethyl)benzylidene) acetohydrazide* ***(4h)***: Light yellow solid, yield 46%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.85, m.p. 238°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.18 (s, 1H, CONH), 7.86–8.07 (m, 4H, quinoxaline ring protons, J = 1.8–7.5 Hz), 8.13–8.17 (q, 4H, A_2B_2 pattern, Ar–H, J = 3–5.1 Hz), 5.32 (s, 2H, CH_2), 5.13 (s, 1H, CH=N), 2.64 (s, 3H, CH_3); IR (KBr, cm^{-1}): 768 (*ortho*-disubstituted aromatic ring), 845 (*para*-disubstituted aromatic ring), 869 (aromatic C=C), 968 (aromatic =C–H, bend), 1199 (=C–N), 1288 (aryl C–F), 1385 (C–N), 1548 (-NH, bend), 1650 (CH=N), 1745 (>C=O), 2344 (N–N=), 3245 (-NH).

2.2m *N′-(2-hydroxybenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4i)***: Light yellow solid, yield 48%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.80, m.p. 248°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.15 (s, 1H, CONH), 7.77–7.87 (m, 4H, quinoxaline ring protons, J = 1.8–6.9 Hz), 7.52–7.53 (d, 1H, Ar–H, J = 4.8 Hz), 7.33–7.35 (d, 1H, Ar–H, J = 7.8 Hz), 7.25–7.28 (t, 2H, Ar–H, J = 4.8–5.7 Hz), 5.52 (s, 2H, CH_2), 5.03 (s, 1H, CH=N), 2.48 (s, 1H, OH), 2.54 (s, 3H, CH_3); IR (KBr, cm^{-1}): 765 (*ortho*-disubstituted aromatic ring), 868 (aromatic C=C), 969 (aromatic =C–H, bend), 1198 (=C–N), 1258 (C–O, str.) 1385 (C–N), 1545 (-NH, bend), 1649 (CH=N), 1748 (>C=O), 2340 (N–N=), 3250 (-NH), 3365 (phenolic -OH).

2.2n *N′-(3-hydroxy-4-methoxybenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4j)***: Yellow solid, yield 66%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.87, m.p. 188°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.12 (s, 1H, CONH), 7.87–7.97 (m, 4H, quinoxaline ring protons, J = 2.1–8.4 Hz), 7.63 (s, 1H, Ar–H), 7.77–7.81 (t, 1H, Ar–H, J = 3–8.2 Hz), 7.82–7.84 (d, 1H, Ar–H, J = 6.6 Hz), 5.22 (s, 2H, CH_2), 5.12 (s, 1H, CH=N), 2.48 (s, 1H, OH), 2.54 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3); IR (KBr, cm^{-1}): 769 (*ortho*-disubstituted aromatic ring), 849 (*para*-disubstituted aromatic ring), 869 (aromatic C=C), 968 (aromatic =C–H, bend), 1045 (C–O–C, *sym*. str.), 1132 (aromatic C–O), 1199 (=C–N), 1245 (C–O–C, *asym*. str.), 1252 (C–O, str.), 1384 (C–N), 1546 (-NH, bend), 1648 (CH=N), 1749 (>C=O), 2345 (N–N=), 3245 (-NH), 3352 (phenolic -OH).

2.2o *N′-(2-fluorobenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4k)***: Red solid, yield 53%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.85, m.p. 248°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.14 (s, 1H, CONH), 7.88–7.98 (m, 4H, quinoxaline ring protons, J = 1.8–7.5 Hz), 7.62–7.65 (d, 1H, Ar–H,

J = 8.4 Hz), 7.43–7.46 (d, 1H, Ar–H, J = 7.5 Hz), 7.55–7.59 (t, 2H, Ar–H, J = 2.4–5.1 Hz), 5.13 (s, 2H, CH_2), 5.23 (s, 1H, CH=N), 2.55 (s, 3H, CH_3); IR (KBr, cm^{-1}): 769 (*ortho*-disubstituted aromatic ring), 864 (aromatic C=C), 968 (aromatic =C–H, bend), 1199 (=C–N), 1285 (aryl C–F), 1387 (C–N), 1548 (-NH, bend), 1650 (CH=N), 1745 (>C=O), 2345 (N–N=), 3248 (-NH).

2.2p *N′-(2-chlorobenzylidene)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide* ***(4l)***: Light yellow solid, yield 43%, $R_{f\,(\text{Benzene : Acetone} = 8:2)}$ 0.80, m.p. 218°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.13 (s, 1H, CONH), 7.78–7.88 (m, 4H, quinoxaline ring protons, J = 1.8–7.8 Hz), 7.52–7.55 (d, 1H, Ar–H, J = 9.9 Hz), 7.33–7.36 (d, 1H, Ar–H, J = 7.8 Hz), 7.45–7.49 (t, 2H, Ar–H, J = 6.6 Hz), 5.03 (s, 2H, CH_2), 5.13 (s, 1H, CH=N), 2.54 (s, 3H, CH_3); IR (KBr, cm^{-1}): 768 (*ortho*-disubstituted aromatic ring), 865 (aromatic C=C), 968 (aromatic =C–H, bend), 1106 (aryl C–Cl), 1198 (=C–N), 1385 (C–N), 1545 (-NH, bend), 1648 (CH=N), 1752 (>C=O), 2346 (N–N=), 3245 (-NH).

2.3 *N-[3-chloro-2-oxo-4-(substituted)phenylazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5a–l)***

Equimolar quantities of compounds (**4a–l**), chloroacetylchloride and triethylamine were dissolved in dry benzene (20 mL) and then stirred continuously at 0°C for about 6 h. Product was filtered, dried and recrystallized with DMF.

2.3a *N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5a)***: Light orange solid, yield 74%, $R_{f\,(\text{TEF})}$ 0.82, m.p. 214°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm): 8.17 (s, 1H, NHCO), 7.77–7.87 (m, 4H, quinoxaline ring protons, J = 1.5–7.8 Hz), 7.52–7.58 (m, 5H, Ar–H, J = 1.2–7.8 Hz), 5.42 (s, 2H, CH_2), 2.556 (s, 3H, CH_3), 3.16 (s, 1H, CH–Cl), 4.10 (s, 1H, CH–Ar); IR (KBr, cm^{-1}): 726 (*mono*-substituted aromatic ring), 765 (*ortho*-disubstituted aromatic ring), 854 (aromatic C=C), 961 (aromatic =C–H, bend), 1195 (=C–N), 1388 (C–N), 1285 (CH–Cl, str.), 1665 (-NCO, str.), 1545 (-NH, bend), 1730 (>C=O), 3244 (-NH); MS (ESI, m/z, M^+): 396.8; Elemental Analysis [Found (Calculated)]: C 60.87 (60.53), H 4.16 (4.32), Cl 9.1 (8.93), N 14.11 (14.12), O 11.76 (12.10).

2.3b *N-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5b)***: Orange solid, yield 78%, $R_{f\,(\text{TEF})}$ 0.84, m.p. 224°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.17 (s, 1H, NHCO), 7.65–7.75 (m, 4H, quinoxaline ring protons, J = 1.2–7.9 Hz), 7.03–7.07 (q, 4H, A_2B_2 pattern, Ar–H, J = 2.7–5.7 Hz), 5.43 (s, 2H, CH_2), 2.54 (s, 3H, CH_3), 3.09 (s, 1H, CH–Cl), 4.14 (s, 1H, CH–Ar); IR (KBr, cm^{-1}): 765 (*ortho*-disubstituted aromatic ring), 824 (*para*-disubstituted aromatic ring), 854 (aromatic C=C), 961 (aromatic =C–H, bend), 1195 (=C–N), 1284 (CH–Cl, str.), 1388 (C–N), 1285 (CH–Cl, str.), 1665 (-NCO, str.), 1545 (-NH, bend), 1677 (-NCO, str.), 1730 (>C=O), 3244 (-NH); MS (ESI, m/z, M^+): 430.7; Elemental Analysis [Found (Calculated)]: C 55.81 (55.70), H 3.65 (3.74), Cl 16.48 (16.44), N 13.02 (12.99), O 11.04 (11.13).

2.3c *N-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5c)***: Biege solid, yield 78%, $R_{f\,(\text{TEF})}$ 0.82, m.p. 234°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.17 (s, 1H, NHCO), 7.65–7.75 (m, 4H, quinoxaline ring protons, J = 1.5–7.5 Hz), 7.33–7.37 (q, 4H, A_2B_2 pattern, Ar–H, J = 1.8–6 Hz), 5.435 (s, 2H, CH_2), 2.54 (s, 3H, CH_3), 3.10 (s, 1H, CH–Cl), 4.16 (s, 1H, CH–Ar), 3.16 (s, 1H, phenolic -OH); IR (KBr, cm^{-1}): 762 (*ortho*-disubstituted aromatic ring), 828 (*para*-disubstituted aromatic ring), 851 (aromatic C=C), 962 (aromatic =C–H, bend), 1199 (=C–N), 1285 (CH–Cl, str.), 1385 (C–N), 1288 (C–O, str., coupled with H–C–H), 1664 (-NCO, str.), 1545 (-NH, bend), 1678 (-NCO, str.), 1729 (>C=O), 3245 (-NH), 3564 (phenolic –OH, str.); MS (ESI, m/z, M^+): 412.5; Elemental Analysis [Found (Calculated)]: C 58.22 (58.19), H 4.20 (4.15), Cl 8.55 (8.59), N 13.58 (13.57), O 15.45 (15.50).

2.3d *N-[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5d)***: Light brown solid, yield 72%, $R_{f\,(\text{TEF})}$ 0.85, m.p. 184°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.22 (s, 1H, NHCO), 7.75–7.85 (m, 4H, quinoxaline ring protons, J = 1.8–8.1 Hz), 7.53–7.57 (q, 4H, A_2B_2 pattern, Ar–H, J = 1.5–5.7 Hz), 5.44 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 3.12 (s, 1H, CH–Cl), 4.16 (s, 1H, CH–Ar),; IR (KBr, cm^{-1}): 763 (*ortho*-disubstituted aromatic ring), 832 (*para*-disubstituted aromatic ring), 849 (aromatic C=C), 959 (aromatic =C–H, bend), 1197 (=C–N), 1285 (CH–Cl, str.), 1368 (aromatic NO_2, *sym.* str.), 1384 (C–N), 1666 (-NCO, str.), 1549 (-NH, bend), 1560 (aromatic NO_2, *asym.* str.), 1679 (-NCO, str.), 1732 (>C=O), 3232 (-NH); MS (ESI,

m/z, M^+): 441.7; Elemental Analysis [Found (Calculated)]: C 54.42 (54.37), H 3.62 (3.65), Cl 7.99 (8.02), N 15.90 (15.85), O 18.07 (18.11).

2.3e *N-[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide **(5e)***: Light yellow solid, yield 78%, $R_{f(TEF)}$ 0.74, m.p. 154°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.10 (s, 1H, NHCO), 7.73–7.81 (m, 4H, quinoxaline ring protons, J = 1.2–7.8 Hz), 7.43–7.47 (q, 4H, A_2B_2 pattern, Ar–H, J = 1.5–5.7 Hz), 5.41 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 3.11 (s, 1H, CH–Cl), 4.13 (s, 1H, CH–Ar), 2.51 (s, 3H, OCH_3); IR (KBr, cm^{-1}): 763 (*ortho*-disubstituted aromatic ring), 829 (*para*-disubstituted aromatic ring), 852 (aromatic C=C), 955 (aromatic =C–H, bend), 1093 (C–O–C, *sym*. str.), 1129 (aromatic C–O, str.), 1199 (=C–N), 1225 (C–O–C, *asym*. str.), 1284 (CH–Cl, str.), 1385 (C–N), 1549 (-NH, bend), 1649 (-NCO, str.), 1681 (-NCO, str.), 1749 (>C=O), 3245 (-NH); MS (ESI, m/z, M^+): 426.3; Elemental Analysis [Found (Calculated)]: C 59.11 (59.09), H 4.45 (4.49), Cl 8.32 (8.31), N 13.16 (13.13), O 14.96 (14.99).

2.3f *N-[3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide **(5f)***: Grey solid, yield 79%, $R_{f(TEF)}$ 0.72, m.p. 144°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 7.99 (s, 1H, NHCO), 7.71–7.79 (m, 4H, quinoxaline ring protons, J = 1.8–7.2 Hz), 7.32 (s, 1H, Ar–H), 7.52–7.57 (t, 1H, Ar–H, J = 1.8–7.5 Hz), 7.42–7.45 (d, 1H, Ar–H, J = 9.3 Hz), 5.40 (s, 2H, CH_2), 2.51 (s, 3H, CH_3), 3.12 (s, 1H, CH–Cl), 4.12 (s, 1H, CH–Ar), 2.56 (s, 6H, OCH_3); IR (KBr, cm^{-1}): 765 (*ortho*-disubstituted aromatic ring), 832 (*para*-disubstituted aromatic ring), 855 (aromatic C=C), 952 (aromatic =C–H, bend), 1094 (C–O–C, *sym*. str.), 1135 (aromatic C–O, str.), 1198 (=C–N), 1232 (C–O–C, *asym*., str.), 1285 (CH–Cl, str.), 1381 (C–N), 1551 (-NH, bend), 1639 (-NCO, str.), 1681 (-NCO, str.), 1752 (>C=O), 3241 (-NH); MS (ESI, m/z, M^+): 456.2; Elemental Analysis [Found (Calculated)]: C 57.85 (57.83), H 4.62 (4.63), Cl 7.80 (7.76), N 12.25 (12.26), O 17.48 (17.51).

2.3g *N-[3-chloro-2-(4-dimethylaminophenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide **(5g)***: Light greenish solid, yield 65%, $R_{f(TEF)}$ 0.78, m.p. 164°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.21 (s, 1H, NHCO), 7.72–7.80 (m, 4H, quinoxaline ring protons, J = 1.8–6.9 Hz), 7.32–7.37 (q, 4H, A_2B_2 pattern, Ar–H, J = 1.8–6.9 Hz), 5.40 (s, 2H, CH_2), 2.518 (s, 3H, CH_3), 3.10 (s, 1H, CH–Cl), 4.12 (s, 1H, CH–Ar), 2.31 (s, 6H, $N(CH_3)_2$); IR (KBr, cm^{-1}): 762 (*ortho*-disubstituted aromatic ring), 832 (*para*-disubstituted aromatic ring), 849 (aromatic C=C), 964 (aromatic =C–H, bend), 1199 (=C–N), 1285 (CH–Cl, str.), 1384 (C–N), 1548 (-NH, bend), 1648 (-NCO, str.), 1680 (-NCO, str.), 1752 (>C=O), 3236 (-NH); MS (ESI, m/z, M^+): 439.3; Elemental Analysis [Found (Calculated)]: C 60.11 (60.07), H 4.99 (5.04), Cl 8.05 (8.06), N 15.99 (15.92), O 10.86 (10.91).

2.3h *N-{3-chloro-2-oxo-4-[4-(trifluoromethyl)phenyl] azetidin-1-yl}-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl) acetamide **(5h)***: Dark brown solid, yield 72%, $R_{f(TEF)}$ 0.78, m.p. 195°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.31 (s, 1H, NHCO), 7.72–7.81 (m, 4H, quinoxaline ring protons, J = 1.8–7.2 Hz), 7.33–7.38 (q, 4H, A_2B_2 pattern, Ar–H, J = 2.7–6.6 Hz), 5.41 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 3.12 (s, 1H, CH–Cl), 4.22 (s, 1H, CH–Ar); IR (KBr, cm^{-1}): 761 (*ortho*-disubstituted aromatic ring), 835 (*para*-disubstituted aromatic ring), 852 (aromatic C=C), 965 (aromatic =C–H, bend), 1198 (=C–N), 1272 (CH–Cl, str.),1288 (aryl C-F, str.), 1386 (C–N), 1548 (-NH, bend), 1649 (-NCO, str.), 1685 (-NCO, str.), 1755 (>C=O), 3245 (-NH); MS (ESI, m/z, M^+): 464.5; Elemental Analysis [Found (Calculated)]: C 54.28 (54.26), H 3.50 (3.47), Cl 7.60 (7.63), F 12.25 (12.26), N 11.99 (12.05), O 10.38 (10.33).

2.3i *N-[3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide **(5i)***: Light brown solid, yield 80%, $R_{f(TEF)}$ 0.81, m.p. 175°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.11 (s, 1H, NHCO), 7.71–7.80 (m, 4H, quinoxaline ring protons, J = 1.8–6.9 Hz), 7.31–7.34 (dd, 2H, Ar–H), 7.41–7.44 (d, 1H, Ar–H, J = 8.4 Hz), 7.37–7.40 (t, 1H, Ar–H, J = 1.5–6 Hz), 5.41 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 3.12 (s, 1H, CH–Cl), 4.22 (s, 1H, CH–Ar), 2.82 (s, 1H, phenolic -OH); IR (KBr, cm^{-1}): 762 (*ortho*-disubstituted aromatic ring), 848 (aromatic C=C), 964 (aromatic =C–H, bend), 1198 (=C–N), 1275 (CH–Cl, str.), 1384 (C–N), 1549 (-NH, bend), 1650 (-NCO, str.), 1685 (-NCO, str.), 1752 (>C=O), 3248 (-NH), 3564 (phenolic –OH, str.); MS (ESI, m/z, M^+): 412.8; Elemental Analysis [Found (Calculated)]: C 58.20 (58.19), H 4.18 (4.15), Cl 8.55 (8.59), N 13.58 (13.57), O 15.49 (15.50).

2.3j *N-[3-chloro-2-(3-hydroxy-4-methoxyphenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5j)***: Grey solid, yield 78%, $R_{f(TEF)}$ 0.77, m.p. 215°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.12 (s, 1H, NHCO), 7.71–7.79 (m, 4H, quinoxaline ring protons, J = 1.5–7.2 Hz), 7.31–7.34 (d, 1H, Ar–H, J = 9 Hz), 7.41–7.44 (d, 1H, Ar–H, J = 9 Hz), 7.37–7.40 (t, 1H, Ar–H, J = 1.5–7.2 Hz), 5.39 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 3.12 (s, 1H, CH–Cl), 4.19 (s, 1H, CH–Ar), 2.81 (s, 1H, phenolic -OH), 3.21 (s, 3H, -OCH_3); IR (KBr, cm^{-1}): 762 (*ortho*-disubstituted aromatic ring), 835 (*para*-disubstituted aromatic ring), 849 (aromatic C=C), 966 (aromatic =C–H, bend), 1192 (=C–N), 1285 (CH–Cl, str.), 1285 (C–O, str., coupled with H–C–H), 1385 (C–N), 1552 (-NH, bend), 1654 (-NCO, str.), 1685 (-NCO, str.), 1754 (>C=O), 3249 (-NH), 3532 (phenolic –OH, str.); MS (ESI, m/z, M^+): 442.9; Elemental Analysis [Found (Calculated)]: C 57.01 (56.95), H 4.33 (4.32), Cl 7.98 (8.01), N 12.69 (12.65), O 17.99 (18.06).

2.3k *N-[3-chloro-2-(2-fluorophenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5k)***: Dark brown solid, yield 68%, $R_{f(TEF)}$ 0.82, m.p. 198°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.22 (s, 1H, NHCO), 7.74–7.82 (m, 4H, quinoxaline ring protons, J = 1.2–6.9 Hz), 7.36–7.39 (dd, 2H, Ar–H), 7.45–7.48 (d, 1H, Ar–H, J = 10.5 Hz), 7.41–7.43 (t, 1H, Ar–H, J = 1.2–7.8 Hz), 5.45 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 3.22 (s, 1H, CH–Cl), 4.26 (s, 1H, CH–Ar); IR (KBr, cm^{-1}): 766 (*ortho*-disubstituted aromatic ring), 852 (aromatic C=C), 962 (aromatic =C–H, bend), 1199 (=C–N), 1272 (CH–Cl, str.), 1285 (aryl C-F, str.),1385 (C–N), 1549 (-NH, bend), 1686 (-NCO, str.), 1752 (>C=O), 3250 (-NH); MS (ESI, m/z, M^+): 414.2; Elemental Analysis [Found (Calculated)]: C 57.93 (57.91), H 3.92 (3.89), Cl 8.51 (8.55), F 4.60 (4.58), N 13.54 (13.51), O 11.50 (11.57).

2.3l *N-[3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide* ***(5l)***: Yellow solid, yield 78%, $R_{f(TEF)}$ 0.85, m.p. 228°C, ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) : 8.21 (s, 1H, NHCO), 7.72–7.81 (m, 4H, quinoxaline ring protons, J = 1.8–7.5 Hz), 7.34–7.38 (dd, 2H, Ar–H), 7.44–7.47 (d, 1H, Ar–H, J = 8.1 Hz), 7.40–7.43 (t, 1H, Ar–H, J = 1.5–6.9 Hz), 5.41 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 3.21 (s, 1H, CH–Cl), 4.25 (s, 1H, CH–Ar); IR (KBr, cm^{-1}): 764 (*ortho*-disubstituted aromatic ring), 846 (aromatic C=C), 965 (aromatic =C–H, bend), 1106 (aryl C-Cl, str.), 1199 (=C–N), 1278 (CH–Cl, str.), 1288 (aryl C-F, str.),1385 (C–N), 1548 (-NH, bend), 1685 (-NCO, str.), 1750 (>C=O), 3229 (-NH); MS (ESI, m/z, M^+): 430.7; Elemental Analysis [Found (Calculated)]: C 55.72 (55.70), H 3.72 (3.74), Cl 16.45 (16.44), N 13.04 (12.99), O 11.09 (11.13).

2.4 *Antibacterial and antifungal activity*

All the synthesized compounds have been screened *in vitro* for antibacterial activity against Gram positive bacteria *S. aureus* (ATCC2913), Gram negative bacteria *K. pneumonia* (ATCC700603), *P. aeruginosa* (ATCC27853), *E. coli* (ATCC25922) and antifungal activity against *C. albicans* (MTCC3017), *A. niger* (MTCC281) and *A. flavus* (MTCC277) by using cup-plate method.

The growth media (Nutrient Agar media for bacterial growth and Sabouraud Dextrose Agar media for fungal growth) were prepared and sterilized in autoclave at 15 psig for 15 min. These media were poured into petri-plates under standard conditions and allowed to solidify. On the surface of media, test microorganisms were inoculated with sterilized nickel loop wire. Cups were made by boring on the surface of growth media with previously sterilized borer. Four cups were made on each petri-plate. These cups were filled with different concentrations (50 μg/mL and 100 μg/mL in DMSO) of the test compounds, third with control (DMSO) and fourth one with standard drug. The plates were kept in cold for 1 h to allow the diffusion of test compounds and then incubated at 35°C for 48 h (for antifungal activity) and at 37°C for 24 h (for antibacterial activity). The zones of inhibition formed around the cups after respective incubation were measured.

3. Results and discussion

3.1 *Chemistry*

Equimolar quantities of pyruvic acid and *ortho*-phenylenediamine were dissolved in 20% HCl and heated with continuous stirring at 45°C for 5 h to give 3-methyl-1H-quinoxaline-2-one **(1)**. The solid residue, thus obtained was purified by treatment with 5% w/v NaOH. Compound **(1)** on refluxing with equimolar quantities of ethylchloroacetate and potassium carbonate in acetone yielded ethyl-[3-methyl-2-oxo-quinoxaline-1-yl]acetate **(2)**. Compound **(2)** on refluxing with hydrazine hydrate in ethanol yielded 3-Methyl-2-oxo-quinoxaline-1(2*H*)-acetohydrazide **(3)**. Compound **(3)** on refluxing with equimolar quantities of appropriate aromatic aldehyde in methanol furnished corresponding Schiff's bases viz. 2-

(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*′-[(substituted) phenylmethylidene]aceto hydrazide **(4a–l)**. Equimolar quantities of compound (**4a–l**), triethylamine and chloroacetylchloride were dissolved in dry benzene and stirred continuously at 0°–5°C for 6 h to yield *N*-[3-chloro-2-oxo-4-(substituted)phenylazetidin-1-yl]-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide **(5a–l)**. Structures of all the newly synthesized compounds were confirmed by their spectral data interpretation.

^{1}H-NMR spectra of 3-methyl-1*H*-quinoxaline-2-one **(1)** showed a doublet at 7.62–7.65 ppm (J = 8.1 Hz) belonging to one proton at C-5 of quinoxaline ring. A triplet in at 7.39–7.44 ppm with J value 5.4–9.6 Hz also confirmed another one proton at C-8 of quinoxaline ring. A multiplet in region 7.18–7.25 ppm (J = 2.7–7.5) confirmed another two protons at C-6 and C-7 of quinoxaline ring. A singlet at 2.36 ppm belonged to three protons of methyl group at C-3 of quinoxaline ring. A singlet at 9.1 ppm confirmed cyclic amide proton. FT-IR spectrum of compound **(1)** showed prominent peaks at 762 cm^{-1}, 1390 cm^{-1}, 1500 cm^{-1}, 1544 cm^{-1}, 1688 cm^{-1}, 3314 cm^{-1} belonging to *ortho*-disubstituted aromatic ring, C–N, aromatic C=C, (-NH (bend)), >C=O, -NH (str.), respectively. ^{1}H-NMR spectrum of ethyl-[3-methyl-2-oxo-quinoxaline-1-yl]acetate **(2)** showed a doublet at 7.73–7.76 ppm (J = 8.1 Hz) belonging to proton at C-5 of quinoxaline ring. A triplet at 7.53–7.55 ppm (J = 3.9–4.2 Hz) belonged to one proton at C-6 of quinoxaline ring. A doublet at 7.43–7.46 ppm (J = 3 Hz) confirmed another proton at C-8 of quinoxaline ring. A triplet at 7.31–7.36 ppm (J = 6.6–8.4) also confirmed another proton at C-7 of quinoxaline ring. A quartlet at 4.11–4.18 ppm confirmed two protons of methylene group of ethoxy group attached to ester linkage at N-1 of quinoxaline ring. A singlet at 3.38 ppm confirmed two methylene protons of CH_2CO group attached at N-1 of quinoxaline ring. A singlet at 2.39 ppm confirmed three protons of methyl group at C-3 of quinoxaline ring. A triplet at 1.14–1.19 ppm confirmed three methyl protons of ethoxy group attached to CH_2CO at N-1 of quinoxaline ring. FT-IR spectrum of compound **(2)** showed prominent peaks at 763 cm^{-1}, 962 cm^{-1}, 1199 cm^{-1}, 1285 cm^{-1}, 1380 cm^{-1}, 1645 cm^{-1}, 1750 cm^{-1}, 2745 cm^{-1} belonging to *ortho*-disubstituted aromatic ring, aromatic =C–H (bend), (=C–N, C–O (coupled with H–C–H)), C–N, CH=N, >C=O, C–C bond respectively. ^{1}H-NMR spectrum of 3-methyl-2-oxo-quinoxaline-1(2*H*)-acetohydrazide **(3)** showed a doublet at 7.72–7.75 ppm (J = 7.8 Hz) belonging to one proton at C-5 of quinoxaline ring. A triplet at 7.49–7.54 ppm (J = 6.3–9 Hz) belonged to another proton at C-8 of quinoxaline ring. A quartlet at 7.26–7.35 ppm (J = 7.5–10.2 Hz) confirmed another two protons at C-6 and C-7 of quinoxaline ring. A singlet at 4.84 ppm belonged to two protons of hydrazine group attached with CH_2CO at N-1 of quinoxaline ring. This anomalous behaviour may due to proton exchange. A singlet at 4.28 ppm belonged to two protons of CH_2CO at N-1 of quinoxaline ring. A singlet at 2.432 ppm confirmed three protons of methyl group at C-3 of quinoxaline ring. FT-IR spectrum of compound **(3)** showed prominent peaks at 760 cm^{-1}, 963 cm^{-1}, 1198 cm^{-1}, 1385 cm^{-1}, 1569 cm^{-1}, 1645 cm^{-1}, 1749 cm^{-1}, 2320 cm^{-1}, 3245 cm^{-1} confirming *ortho*-disubstituted aromatic ring, aromatic =C–H, bend, =C–N, C–N, -NH (bend), CH=N, >C=O, N–N, -NH (str.), respectively. ^{1}H-NMR spectrum of N′-benzylidene-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetohydrazide **(4a)** showed a singlet at 8.06 ppm belonging to proton of amide linkage. A multiplet at 7.67–7.77 ppm (J = 3–7.5 Hz) confirmed four quinoxaline protons at C-5, C-6, C-7 and C-8. A multiplet at 7.41–7.53 ppm (J = 2.7–8.1 Hz) confirmed five aromatic protons of phenyl group attached to imine linkage. A singlet at 5.42 ppm confirmed two protons of CH_2CO at N-1 of quinoxaline ring. A singlet at 5.03 ppm confirmed proton of imine linkage with CH_2CO group. A singlet at 2.54 ppm confirmed three protons of methyl group attached to C-3 of quinoxaline ring. FT-IR spectrum of compound **(4a)** showed prominent peaks at 723 cm^{-1}, 769 cm^{-1}, 854 cm^{-1}, 964 cm^{-1}, 1199 cm^{-1}, 1388 cm^{-1}, 1542 cm^{-1}, 1650 cm^{-1}, 1729 cm^{-1}, 2345 cm^{-1}, 3244 cm^{-1} confirming *mono*-substituted aromatic ring, *ortho*-disubstituted aromatic ring, aromatic C=C, aromatic =C–H (bend), =C–N, C–N, -NH (bend), CH=N, CH=N, >C=O, N–N=, -NH (str.), respectively. ^{1}H-NMR spectrum of *N*-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide **(5a)** showed a singlet at 8.17 ppm belonging to amide group. A multiplet at 7.77–7.87 ppm (J = 1.5–7.8 Hz) confirmed four quinoxaline protons at C-5, C-6, C-7 and C-8. A multiplet at 7.52–7.58 ppm belonged to five aromatic protons of phenyl group attached to C-4 of azetidinone ring. A singlet at 5.42 ppm belonged to two protons of CH_2CO attached to N-1 of quinoxaline ring. A singlet at 2.55 ppm belonged to three protons of CH_3 at C-3 of quinoxaline ring. A singlet at 3.16 ppm confirmed one proton at C-3 of azetidinone ring, while another singlet at 4.10 ppm confirmed one proton at C-4 of azetidinone ring. FT-IR spectrum of compound **(5a)** showed prominent peaks at 726 cm^{-1}, 765 cm^{-1}, 854 cm^{-1}, 961 cm^{-1}, 1195 cm^{-1}, 1388 cm^{-1}, 1285 cm^{-1}, 1665 cm^{-1}, 1545 cm^{-1}, 1730 cm^{-1}, 3244 cm^{-1} confirming *mono*-substituted aromatic ring, *ortho*-disubstituted aromatic ring,

aromatic C=C, aromatic =C–H (bend), =C–N, C–N, CH–Cl (str.), -NCO (str.), -NH (bend), >C=O, -NH (str.), respectively. ESI-MS of compound **(5a)** showed M^{*+} at m/z 396.8. Similarly, structures of all the newly synthesized were confirmed.

3.2 *Biological activity*

All the newly synthesized compounds have been evaluated for their antibacterial activity against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and antifungal activity against *C. albicans*, *A. niger* and *A. flavus*. In the present communication, we have concentrated on 'the effect of various substituents on phenyl ring attached at 4-position of azetidinone nucleus attached to N-atom of acetamido functionality on 1-position of 3-methyl-2-oxo-quinoxaline-1(2*H*)-acetamide'.

Against *E.coli*, compounds with 4-Cl **(5b)**, 4-NO_2 **(5d)**, 4-$N(CH_3)_2$ **(5g)**, 4-CF_3 **(5h)**, 3-OH-4-OCH_3 **(5j)** on phenyl ring attached to 4-position of azetidinone ring on the above said position have shown better activity, while compounds with functionalities unsubstituted phenyl ring **(5a)**, 4-OH **(5c)**, 4-OCH_3 **(5e)**, 3,4-$(OCH_3)_2$ **(5f)**, 2-OH **(5i)**, 2-F **(5k)** on the phenyl ring attached to azetidinone ring have exhibited moderate activity. Among azetidinone derivatives, compounds with 4-Cl **(5b)**, 4-OH **(5c)**, 4-NO_2 **(5d)**, 4-$N(CH_3)_2$ **(5g)**, 4-CF_3 **(5h)**, 3-OH-4-OCH_3 **(5j)** on the phenyl ring attached at above mentioned position have shown better activity against *S. aureus*. While, compounds with 4-OCH_3 **(5e)**, 3,4- $(OCH_3)_2$ **(5f)**, 2-OH **(5i)**, 2-F **(5k)**, 2-Cl **(5l)** exhibited intermediate activity against *S. aureus*. Against *K. pneumoniae*, compounds with functional groups 4-Cl **(5b)**, 4-OH **(5c)**, 4-NO_2 **(5d)**, 4-$N(CH_3)_2$ **(5g)**, 4-CF_3 **(5h)** on phenyl ring attached to 4-position of azetidinone ring exhibited better activity. Compounds **5i** having 2-OH-C_6H_4 on 4-position of azetidinone ring has shown minimal activity against *K. pneumoniae*. Most of newly synthesized compounds have shown good antibacterial activity against *P. aeruginosa*. Compounds having 4-Cl **(5b)**, 4-OH **(5c)**, 4-NO_2 **(5d)**, 4-$N(CH_3)_2$ **(5g)**, 4-CF_3 **(5h)**, and 3-OH-4-OCH_3 **(5j)** on phenyl ring attached to above mentioned positions at azetidinone ring exhibited better activity. Against fungal strains *C. albicans*, *A. niger*, *A. flavus*, most of the newly synthesized compound exhibited moderate activity. Azetidinone derivatives with 4-OH **(5c)**, 4-$N(CH_3)_2$ **(5g)**, 4-CF_3 **(5h)** and 3-OH-4-OCH_3 **(5j)** groups on phenyl ring have been found to be more active, while, compound **5l** having 2-Cl-C_6H_4 group on respective position on azetidinone nucleus exhibited minimal activity. Against, *A. niger*, compounds **5g** and **5j** having 4-$N(CH_3)_2$-C_6H_4 and 3-OH-4-OCH_3-C_6H_3 groups on 4-position of azetidinone ring exhibited better activity. Azetidinone derivatives with 4-$N(CH_3)_2$-C_6H_4 **(5g)** and 4-CF_3-C_6H_4 **(5h)** on 4-position of azetidinone ring showed better activity while compounds **5i**, **5k** and **5l** having 2-OH-C_6H_4, 2-F-C_6H_4 and 2-Cl-C_6H_4, respectively exhibited minimal activity.

Compounds **5b**, **5d**, **5g** and **5h** having 4-Cl, 4-NO_2, 4-$N(CH_3)_2$ and 4-CF_3, respectively on phenyl ring attached to 4-position of azetidinone ring attached to N-atom of acetamido group on 1-position of 3-methyl-2-oxo-quinoxaline-1(2*H*)-acetamide were found to be more active against all the bacterial strains under investigation. Compound **5g** was also found to be more active against all bacterial and fungal strains under investigation.

4. Conclusion

All the synthesized compounds were evaluated for their antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and antifungal activity against *C. albicans*, *A. niger* and *A. flavus* using cup-plate method. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)acetamide-based azetidinone derivatives having aromatic phenyl ring substituted with 4-Cl, 4-NO_2, 4-$N(CH_3)_2$ and 4-CF_3 exhibited better activity against all the bacterial strains under investigation. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)acetamide-based azetidinone derivatives having aromatic phenyl ring substituted with 4-$N(CH_3)_2$ **(5g)** was found to be most active against all bacteria and fungi under investigation. These compounds may act as lead for future investigation.

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