Research Article

Microwave-Assisted Synthesis of Some Quinoxaline-Incorporated Schiff Bases and Their Biological Evaluation

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Quinoxaline-incorporated Schiff bases (**4a-j**) were synthesized by the condensation of 2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide (**3**) with indole-3-carbaldehyde, furfuraldehyde, 5-(4-nitrophenyl)-2-furfuraldehyde, and substituted benzaldehydes under conventional and microwave irradiation methods. The microwave method was found to be remarkably successful with higher yields, less reaction time, and environmentally friendly compared to conventional heating method. The chemical structures of the synthesized compounds have been confirmed by analytical and spectral data. All the compounds have been evaluated for antitubercular and anti-inflammatory activities.

1. Introduction

Quinoxaline derivatives have been reported to possess a wide range of biological properties such as being anticancer [1, 2], antihistaminic [3], antiviral [4], antimicrobial [5, 6], antifungal [7], antitubercular [8, 9], and anti-inflammatory [10]. Schiff bases possess antimicrobial [11], antitubercular [12], anti-inflammatory [13], and analgesic [14] properties. In view of these facts, we herein report the facile synthesis of a new series of quinoxaline-incorporated schiff bases and screen for antitubercular and anti-inflammatory activities. In recent times, microwave-assisted organic reactions have become very popular and gain special attention due to their ecofriendly nature, safety, less reaction time, and higher yields. In continuation of our research on quinoxaline derivatives, in the present study, we made an attempt to synthesize the title compounds using conventional and microwave irradiation methods and compared the reaction time and percentage yields.

2. Experimental

Melting points were determined using open capillary tubes on ANALAB melting point apparatus and are uncorrected. IR spectra were recorded on FTIR spectrophotometer (SHI-MADZU 8400 series) using KBr pellets. ^1H NMR spectra

were recorded in $\text{CDCl}_3/\text{DMSO}-d_6$ solvents unless otherwise mentioned. Chemical shifts are reported on AVANCE 300 MHz and INNOVA 400 MHz relative to TMS internal standard on the δ -scale. Mass spectra of the compounds were recorded on Mass spectrometer (Agilent 1100 series; EI/ES-MS) at Indian Institute of Chemical Technology, Hyderabad. The syntheses were carried out in scientific microwave oven supplied by Catalyst, India. All the reactions were monitored by thin layer chromatography on 60 F₂₅₄ 0.25 mm precoated aluminum plates (Merck) and visualized with UV light.

3. Results and Discussion

3-methylquinoxalin-2-ol (**1**) was synthesized by condensing o-phenylenediamine with ethyl pyruvate. Compound (**1**) was converted into ethyl [(3-methylquinoxalin-2-yl)oxy]acetate (**2**) by heating under reflux with ethyl chloroacetate in dry acetone and anhydrous potassium carbonate. The structure of compound (**2**) was confirmed by the carbonyl peak of ester at 1740 cm^{-1} in IR, and a mass ion peak at m/z 247 (100%) in mass spectroscopy. The reaction of the compound (**2**) with hydrazine hydrate (99%) in ethanol under reflux for 5-6 hr gave 2-[(3-methylquinoxaline-2-yl)oxy]acetohydrazide (**3**). The carbonyl peak of amide at 1680 cm^{-1} and NH, NH_2 stretching at 3330 cm^{-1} and 3226 cm^{-1} in IR and a mass

SCHEME 1

ion peak at m/z 233 (100%) corresponding to its molecular weight in mass spectrum confirmed the structure of the compound (3). From compound (3), ten different Schiff bases have been synthesized (Scheme 1) by condensing with indole-3-carbaldehyde, furfuraldehyde, 5-(4-nitrophenyl)-2-furfuraldehyde, and substituted benzaldehydes using conventional and microwave irradiation methods. The physical data of Schiff bases, time and yields in both the methods, were compared and mentioned in the Table 1.

3.1. Antitubercular Activity. All the newly synthesized compounds were evaluated for their possible *in vitro* antitubercular activity at a concentration of $6.25 \mu\text{g/mL}$ against *Mycobacterium tuberculosis* $H_{37}RV$ (ATCC 27294) in BACTEC 12B medium using both micro dilution assay and the microplate alamar blue assay (MABA). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. The antitubercular activity data were compared with standard drug, Rifampin at a concentration of $0.25 \mu\text{g/mL}$. The compounds 4c, 4d, 4i, and 4j exhibited substantial antitubercular activity, particularly 4h showed 75% inhibition

(MIC > $6.25 \mu\text{g/mL}$) and emerged as the most active compound in the present series. The MIC and percentage inhibition data of the compounds that have shown activity against *Mycobacterium tuberculosis* are presented in Table 2.

3.2. Anti-Inflammatory Activity. Male Wistar rats (140–200 g) of both sexes were used for the studies. The rats were obtained from Mahaveer enterprises, Hyderabad. The animals were divided into groups of six each and fasted for 12 hr before the experiment. The ethical guidelines prescribed for the investigation of animals used in experiments were followed in all tests.

Carrageenan (0.1 mL, 1%) was administered into the plantar surface of the right hind paw of each rat, 1 hr after the administration of the test compounds and standard drug ibuprofen (100 mg/kg). One group was kept as control, received only 0.5% CMC solution. Before injection of carrageenan, the average volume (V_o) of the right hind paw of each rat was calculated. After injection, the paw volume (V_t) was measured after 3rd hr with the aid of a plethysmograph. The edema was expressed as an increase in the volume of paw,

TABLE 1: Characterization data of compounds **4(a-j)**.

Comd	Ar	M.P (°C)	Conventional Time(hr)	Yield (%)	Microwave Time (min)	Yield (%)
4a	C ₆ H ₅	278	6	75	7	90
4b	4-Cl-C ₆ H ₄	260	5	69	6	80
4c	3-NO ₂ -C ₆ H ₄	289	7	80	7	92
4d	4-OH-C ₆ H ₄	246	6	65	7	81
4e	3,4-(OCH ₃) ₂ -C ₆ H ₃	240	6	76	8	85
4f	4-OCH ₃ -C ₆ H ₄	281	7	72	7	89
4g	4-N(CH ₃) ₂ -C ₆ H ₄	274	6	66	6	83
4h	3-indolyl	256	7	60	7	82
4i	2-furyl	295	5	68	6	84
4j	5-(4-nitrophenyl)-2-furfuryl	232	6	74	8	91

TABLE 2: Percentage inhibition of compounds against *Mycobacterium tuberculosis* H₃₇RV at MIC (6.25 µg/mL) **4(a-j)**.

Comd	% Inhibition
4a	23
4b	15
4c	66
4d	58
4e	25
4f	10
4g	32
4h	75
4i	56
4j	50
Rifampin	95

and percentage inhibition of acute edema was obtained as follows:

$$\% \text{ Inhibition} = \left[1 - \left(\frac{\Delta V_{\text{experimental}}}{\Delta V_{\text{control}}} \right) \right] \times 100, \quad (1)$$

where

$$\Delta V = V_t - V_o = \text{Mean paw volume}. \quad (2)$$

Among all the compounds, compounds **4a**, **4b**, **4d**, **4g**, **4h** and **4i** exhibited significant activity at 3rd hr (Table 3). Results are presented as Mean ± SEM (standard error of mean) of six rats. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons, using Graph-pad Software.

3.3. Toxicity Study. Acute toxicity of schiff bases was determined in albino mice with the staircase method. Each group of 5 animals was fasted for 24 hr prior to the administration of the test compounds. The test compounds, **4a**, **4b**, **4d**, **4g**, **4h**

TABLE 3: *In vivo* anti-inflammatory activities of Schiff bases.

Comd	$V_t - V_o$ [Mean ± SEM]	% Inhibition of edema at the end of 3rd hr
4a	0.42 ± 0.011	55.3
4b	0.32 ± 0.004	65.9
4d	0.38 ± 0.005	59.5
4g	0.34 ± 0.003	63.8
3h	0.33 ± 0.004	64.8
4i	0.32 ± 0.004	65.9
Ibuprofen	0.26 ± 0.018	73.5

Values expressed as Mean ± SEM, $n = 6$ in each group $P > 0.01$ compared with control.

and **4i** were administered orally in doses up to 2000 mg/kg and mice were kept under observation for period of 24 hrs.

4. General Procedure for the Synthesis of Schiff Bases

4.1. Microwave Irradiation Method. 2-[(3-Methylquinoxalin-2-yl)oxy]acetohydrazide (**3**) (0.01 mole, 2.32 g) was dissolved in 10 mL of DMF and to this was added heterocyclic/aromatic aldehyde (0.01 mole) and few drops of glacial acetic acid. The mixture was transferred to a vessel and kept in the microwave oven. The oven was run at 400–480 W for different time for different reaction mixtures. The completion of the reaction was monitored continuously by TLC after every minute. The product obtained was poured into water, filtered, dried, and recrystallized from a mixture of DMF and water.

4.2. Conventional Method. Equimolar mixture of 2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide (**3**) and appropriate heterocyclic/aromatic aldehyde was dissolved in 90% ethanol and to this was added catalytic amount of glacial acetic acid and refluxed for 5–7 hr. Excess ethanol was removed under reduced pressure, and the residue was purified by washing with cold ethanol and recrystallized from DMF and water mixture.

2-[(3-Methylquinoxalin-2-yl)oxy]-N'-[(1E)-phenylmethylene]acetohydrazide **4a**. Colorless solid; IR (KBr): 1656.74 (C=N str), 1679.88 (C=O str), 2958.6 (C-H str of CH₃); ¹H NMR (CDCl₃): δ 11.84 (s, 1H, NH), 8.08 (s, 1H, CH=N), 7.77–7.36 (m, 9H, Ar-H), 5.46 (s, 2H, OCH₂), 2.48 (s, 3H, CH₃); Mass (M + H)⁺: m/z 321 (100%). Anal. Calcd for C₁₈H₁₆N₄O₂: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.33; H, 5.01; N, 17.32%.

N'-[(1E)-(4-Chlorophenyl)methylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4b**. Colorless solid; IR (KBr): 1654.81 (C=N str), 1685.67 (C=O str), 2958.6 (C-H str of CH₃), 754.12 (C-Cl); ¹H NMR (CDCl₃): δ 11.92 (s, 1H, NH), 8.23 (s, 1H, CH=N), 7.89–7.47 (m, 8H, Ar-H), 5.54 (s, 2H, OCH₂), 2.81 (s, 3H, CH₃); Mass (M + H)⁺: m/z 355 (100%). Anal. Calcd for C₁₈H₁₅ClN₄O₂: C, 60.94; H, 4.26; N, 15.79. Found: C, 60.73; H, 4.15; N, 15.74%.

2-[(3-Methylquinoxalin-2-yl)oxy]-N'-[(1E)-(3-nitrophenyl)methylene]acetohydrazide **4c**. Pale yellow solid; IR (KBr): 1654.81 (C=N str), 1689.53 (C=O str), 2948.96 (C-H str of CH₃), 1320, 1480 (NO₂ str); ¹H NMR (CDCl₃): δ 11.96 (s, 1H, NH), 8.42 (s, 1H, CH=N), 7.98–7.53 (m, 8H, Ar-H), 5.67 (s, 2H, OCH₂), 2.92 (s, 3H, CH₃); Mass (M + H)⁺: m/z 366 (100%). Anal. Calcd for C₁₈H₁₅N₅O₄: C, 59.18; H, 4.14; N, 19.17. Found: C, 59.13; H, 4.11; N, 19.12%.

N'-[(1E)-(4-Hydroxyphenyl)methylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4d**. Colorless solid; IR (KBr): 1658.67 (C=N str), 1681.81 (C=O str), 2958.6 (C-H str of CH₃), 3352.05 (O-H str); ¹H NMR (CDCl₃): δ 11.64 (d, J = 10.2, NH), 9.94 (s, 1H, OH), 7.97 (s, 1H, N=CH), 7.78–6.83 (m, 8H, Ar-H), 5.41 (s, 2H, OCH₂), 2.47 (s, 3H, CH₃); Mass (M + H)⁺: m/z 337 (100%). Anal. Calcd for C₁₈H₁₆N₄O₃: C, 64.28; H, 4.79; N, 16.66. Found: C, 64.13; H, 4.71; N, 16.56%.

N'-[(1E)-(3,4-Dimethoxyphenyl)methylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4e**. Pale brown solid; IR (KBr): 1647.1 (C=N str), 1677.95 (C=O str), 2948.96 (C-H str of CH₃), 1267.14 (C-O-C str); ¹H NMR (CDCl₃): δ 11.23 (s, 1H, NH), 8.13 (s, 1H, CH=N), 7.83–7.47 (m, 7H, Ar-H), 5.48 (s, 2H, OCH₂), 3.82 (s, 6H, OCH₃), 2.43 (s, 3H, CH₃); Mass (M + H)⁺: m/z 381 (100%). Anal. Calcd for C₂₀H₂₀N₄O₄: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.07; H, 5.28; N, 14.62%.

N'-[(1E)-(4-Methoxyphenyl)methylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4f**. Colorless solid; IR (KBr): 1654.81 (C=N str), 1679.88 (C=O str), 2962.46 (C-H str of CH₃), 1257.50 (C-O-C str); ¹H NMR (CDCl₃): δ 11.53 (s, 1H, NH), 8.24 (s, 1H, CH=N), 7.82–7.58 (m, 8H, Ar-H), 5.53 (s, 2H, OCH₂), 3.88 (s, 3H, OCH₃), 2.51 (s, 3H, CH₃); Mass (M + H)⁺: m/z 351 (100%). Anal. Calcd for C₁₉H₁₈N₄O₃: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.10; H, 5.06; N, 15.63%.

N'-[(1E)-[4-(Dimethylamino)phenyl]methylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4g**. Colorless solid; IR (KBr): 1654.81 (C=N str), 1677.95 (C=O str), 2920 (C-H str of CH₃), 1365.51 (C-N str); ¹H NMR (CDCl₃): δ 11.16 (s, 1H, NH), 8.15 (s, 1H, CH=N), 7.75–7.38 (m, 7H, Ar-H), 5.32 (s, 2H, OCH₂), 3.19 (s, 6H, N(CH₃)₂), 2.32 (s, 3H, CH₃); Mass (M + H)⁺: m/z 364 (100%). Anal. Calcd for

C₂₀H₂₁N₅O₂: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.05; H, 5.66; N, 19.23%.

N'-[(1E)-1H-Indol-3-ylmethylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4h**. Pale brown solid; IR (KBr): 1650.95 (C=N str), 1677.95 (C=O str), 2991.39 (C-H str of CH₃), 3263.33 (N-H str of Ind-NH); ¹H NMR (CDCl₃): δ 11.58 (d, J = 19.8, 2H, Ind-H, NH), 8.268 (s, 1H, CH=N), 8.17–7.15 (m, 9H, Ar-H), 5.5 (s, 2H, OCH₂), 2.47 (s, 3H, CH₃); Mass (M + H)⁺: m/z 360 (100%). Anal. Calcd for C₂₀H₁₇N₅O₂: C, 66.84; H, 4.77; N, 19.49. Found: C, 66.75; H, 4.66; N, 19.39%.

N'-[(1E)-2-Furylmethylene]-2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **4i**. Pale yellow solid; IR (KBr): 1660.6 (C=N str), 1683.74 (C=O str), 2954.74 (C-H str of CH₃), 1288.36 (C-O str); ¹H NMR (CDCl₃): δ 11.77 (d, J = 7.6, 1H, NH), 7.96 (s, 1H, CH=N), 7.84–6.64 (m, 7H, Ar-H), 5.36 (s, 2H, OCH₂), 2.46 (s, 3H, CH₃); Mass (M + H)⁺: m/z 311 (100%). Anal. Calcd for C₁₆H₁₄N₄O₃: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.85; H, 4.66; N, 18.35%.

2-[(3-Methylquinoxalin-2-yl)oxy]-N'-[(1E)-[5-(4-nitrophenyl)-2-furfuryl]methylene]acetohydrazide **4j**. Yellow solid; IR (KBr): 1654.61 (C=N str), 1689.4 (C=O str), 2956.24 (C-H str of CH₃), 1286.53 (C-O str); ¹H NMR (CDCl₃): δ 11.69 (s, 1H, NH), 7.84 (s, 1H, CH=N), 7.78–6.73 (m, 10H, Ar-H), 5.24 (s, 2H, OCH₂), 2.53 (s, 3H, CH₃); Mass (M + H)⁺: m/z 432 (100%). Anal. Calcd for C₂₂H₁₇N₅O₅: C, 61.25; H, 3.97; N, 16.23. Found: C, 61.18; H, 3.68; N, 16.15%.

5. Conclusion

A novel series of schiff bases were synthesized by using conventional and microwave irradiation methods and all the compounds were characterized by physical and spectral data. Compounds **4c**, **4d**, **4h**, **4i**, and **4j** exhibited potent antitubercular activity. Compounds **4a**, **4b**, **4d**, **4g**, **4h**, and **4i** have shown significant protection against the edema formation. With further molecular modification and manipulation of these compounds, several other promising bioactive molecules can be developed in future.

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