Synthesis and Evaluation of N'-((Substituted Phenyl) Methyldene)-2-(3-Methyl-2-oxyquinoxalin-1 (2H)-yl)Acetohydrazide for Possible Antimicrobial and Antifungal Activities

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Abstract: A novel synthetic methodology of Schiff’s bases incorporating 3-methylquinoxalin-2(1H)-one is described. The title compounds were prepared by condensation of substituted aromatic aldehydes and 2-(3-methyl-2-oxyquinoxalin-1(2H)-yl) acetic acid hydrazide. Structures of all these compounds were confirmed by their spectral studies. These compounds were screened for in vitro antitubercular, antibacterial and antifungal activities. From the biological studies, it was possible to observe that some of the substituent on the phenyl ring of quinoxalinone hydrazones influenced the activity. Among synthesized compounds (4f, 4g, 4i and 4j), have shown good anti tubercular activity (25 \( \mu \)g mL\(^{-1}\)) when compared to reference drug. Compounds (4g and 4j) showed moderate to good antimicrobial activity at low concentration. The MICs (Minimum Inhibitory Concentration) against gram positive, gram negative and some species of fungi are in the range 2-4 \( \mu \)g mL\(^{-1}\) when compared to standard drug. In conclusion, the antimicrobial testing results revealed that the compounds possess broad spectrum of in vitro antimicrobial activity at low concentration. The ambient conditions, excellent product yields and easy workup procedures make this methodology a better protocol for the synthesis of newer derivatives.

Key words: 3-Methylquinoxalin-2-one-N'-(sub-phenyl) methylidene, Schiff’s bases, antimycobacterial activity, antibacterial activity, antifungal activity, minimum inhibitory concentration

INTRODUCTION

Tuberculosis is an ancient killer infectious disease that has plagued humans for centuries and it remained a leading infectious disease worldwide today (Yves, 2007). As resistant strains of Mycobacterium tuberculosis have slowly emerged, treatment failure is too often a fact especially in countries lacking the necessary health care organization to provide the long and costly treatment adapted to patients. Due to this concern, this infectious disease was the focus of renewed scientific interest in the last decade. According to World Health Organization (WHO) report currently, one third of the world populations about 2 billion people are latently infected with tuberculosis (TB) bacteria (Stram et al., 2006). There are 8 million new TB cases worldwide with 2 million deaths every year (Zhang and Amzel, 2002).

In continuation of our study, we report the synthesis of novel Schiff’s bases incorporating quinoxaline moiety. Quinoxalines constitutes an important class of compounds, some analogs are synthesized and evaluated for antimicrobial activity (Ali et al., 2000; Mohsenzadeh et al., 2007) and
many possess diverse biological activity such as insecticides, fungicides, herbicides, anthelmintics and antiviral (Rafat et al., 2004; Vyak et al., 2007). Among the various classes of nitrogen heterocyclic compounds, quinazoline derivatives display a broad spectrum of biological activities. This has contributed to their usefulness in combinatorial drug discovery libraries (Heravi et al., 2009). Synthetic quinazoline ring is the part of a number of antibiotics which are known to inhibit the growth of gram positive bacteria and are also active against Mycobacterium tuberculosis. In addition, Schiff’s bases are also known for their antibacterial and antitubercular activity (Verughp et al., 2007; Rao et al., 2007; Aboul-Fadl et al., 2003). In the present study, we describe the synthesis of novel Schiff’s bases incorporating quinazoline moieties. Title compounds were screened for their in vitro antibacterial, antifungal and antitubercular activity by broth dilution method. In the study of its biological activity against selected strains of bacteria and fungi, it has been investigated for their Minimum Inhibitory Concentration (MIC).

MATERIALS AND METHODS

Melting points were determined in open capillaries on a Thermon melting point apparatus and were found uncorrected. Infr Red spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu FT-IR 8700) using KBr (νmax in cm⁻¹) disc method. 1H NMR spectra were recorded in CDCl₃ and DMSO on Bruker-200 NMR spectrophotometer using TMS as internal reference standard (chemical shifts in δ, ppm). Mass spectra were recorded on Shimadzu 2010A LCMS spectrophotometer (m/z and relative intensity). All the reactions were routinely monitored and purity was determined on thin layer chromatography using Merk silica gel 60 F₂₅₄ coated aluminium plates using several solvent systems of different polarity. All the chemicals used were of AR grade (Sigma-Aldrich; Acris; Hi-media). Minimum Inhibitory Concentration (MIC) was determined by broth dilution method where Mueller-Hinton agar, Middle brock 7H-9 broth and Sabouraud’s Dextrose agar media were used for MIC determination.

This study was carried out from 15th January 2008 to 15th December 2008 at Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bangalore-560 027, India and Department of Microbiology, M.M’s N. G. Halgekar Institute of Dental Sciences and Research Center, Belgaum, India.

Tested Microorganism

Laboratory isolates of pure cultures of gram negative (Klebsiella pneumonia ATCC 29665, Escherichia coli ATCC 25922) and gram positive (Staphylococcus aureus ATCC 12598, Enterococcus fecalis ATCC 55550) bacteria. Candida albicans ATCC 2091 and Aspergillus flamigates ATCC 13073 representative fungi and Mycobacterium tuberculosis H₃₇Rv were obtained from Department of Microbiology, M.M’s N. G. Halgekar Institute of Dental Sciences and Research Center, Belgaum, India.

Synthesis of 3-methylquinazalin-2(1H)-one (1)

Compound (1) was prepared according to the method described by Krishnan et al. (2001). To a solution of o-phenylenediamine (5.4 g, 0.05 mol) in water (60 mL), a solution of pyruvic acid (3.5 mL, 0.05 mol) in water (20 mL) was added. The mixture was stirred at room temperature for 15 min. At the end of this period, the product separated was filtered, washed with water (2×20 mL) and dried. The crude product was purified by dissolving in aqueous sodium hydroxide (5% w/v, 30 mL) followed by charcoal treatment and filtration. The filtrate was cooled and neutralized with acetic acid to a pH of 6.0. The separated product was filtered and washed with water (2×20 mL). The product was reccrystallised from alcohol to light yellow needles with yield 5.5 g (68%) mp 249-251°C (Lit 248-250°C).
Synthesis of Ethyl 2-(3-Methyl-2-oxoquinazolin-1(2H)-yl)acetate (2)

This compound was prepared as per the procedure described by Mogilaiah et al. (2004). A mixture of 3-methylquinazolin-2(1H)-one (2.32 g, 0.01 mol), ethyl chloroacetate (1.22 g, 0.01 mol), anhydrous K$_2$CO$_3$ (1.38 g, 0.01 mol) and dimethylformamide (15 mL) was stirred at room temperature for 8 h. The reaction mixture was diluted with ice-cold water. The separated solid was filtered, washed with water and recrystallised from cyclohexane to give colorless needles with yield 2.49 g (70%), m.p. 128-30°C:

- IR (KBr, νmax in cm$^{-1}$): 3115 (Ar-H), 1664 (ring C = O), 1550 (Ar-C = C), 1533 (C = N)
- $^1$H NMR (CDCl$_3$, δ ppm): 1.28 (t, 3H, CH$_3$), 1.61 (s, 3H, ring CH$_3$), 4.27 (q, 2H, CH$_2$) 5.02 (s, 2H, N-CH$_3$), 7.04-7.85 (m, 4H, Ar-H)
- LCMS, m/z$: 245(M^+1, 80%)$, 231 (5), 217 (15), 199 (10) and 159 (10)

Synthesis of 2-(3-methyl-2-oxoquinazolin-1(2H)-yl)acetic acid hydrazide (3)

A solution of ethyl 2-(3-methyl-2-oxoquinazolin-1(2H)-yl)acetate (2.46 g, 0.01 mol) and hydrazine hydrate (0.75 g, 0.015 mol) in ethanol (15 mL) was refluxed for 4 h. The reaction mixture was cooled and poured onto ice-cold water with stirring. The separated solid was filtered, washed with water and recrystallised from ethanol to afford (3), yield 1.9 g (81.8%), m.p. 218-20°C:

- IR (KBr, νmax in cm$^{-1}$): 3332, 3209 (-NHNH$_2$, str), 1685 (ring C = O), 1654 (CONH), 1600 (C = N)
- $^1$H NMR (DMSO, δ ppm): 2.44 (s, 2H, N-CH$_3$), 3.42 (s, 3H, ring CH$_3$) 4.95(br 2H-NH$_2$) 7.21-7.78 (m, 4H, Ar-H), 9.37 (s, 1H, CONH)
- LCMS, m/z$: 233 (M+1, 50%)$, 203 (100), 175 (60), 160 (40) and 145 (20)

Synthesis of N’-(Substituted phenyl)methylidene-2-(3-methyl-2-oxoquinazolin-1(2H)-yl)acetohydrazide (4a-4j)

General procedure: A mixture of 2-(3-methyl-2-oxoquinazolin-1(2H)-yl)acetic acid hydrazide (1.16 g, 0.005 mol) and substituted aromatic aldehyde (4a-4j) (0.005 mol) in dimethylformamide (10 mL) was refluxed for 5 h, cooled and poured onto water (100 mL) containing crushed ice. The precipitate thus obtained was filtered, washed with cold water and recrystallised from DMSO-water mixture to obtain Schiff bases (4a-4j) (Mogilaiah et al., 2004). Similar synthetic method was adopted to prepare title compounds and the results are tabulated in Table 1.

Physical and spectral data of N’-(2-nitrophenyl)methylidene-2-(3-methyl-2-oxoquinazolin-1(2H)-yl)acetohydrazide (4i)

- Yield 1.55 g (85%), m.p. 260-62°C
- IR (KBr, νmax in cm$^{-1}$): 1677 (ring C = O), 1645 (CONH), 1600 (C = N)
- $^1$H NMR (DMSO, δ ppm): 2.50 (s, 3H, ring CH$_3$), 5.46(s, 1H, CONH), 7.33-8.47 (m, 8H, Ar-H), 8.65(s, 1H, N = CH)
- LCMS, m/z$: 365(M+1, 60%)$, 282 (100), 201 (80) and 161 (15)

Antimonycobacterial Activity

Test compounds were evaluated for in vitro antimonycobacterial activity. The MICs were determined and interpreted for Mycobacterium tuberculosis H$_3$Rv according to the procedure of the approved macrodilution reference method of antimicrobial susceptibility testing (Andrews, 2001).
Table 1: Characterization data of compounds (1-3 and 4a-4j)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Mol. formula (Mol. weight)</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt; (160)</td>
<td>249-51</td>
<td>69</td>
<td>0.65</td>
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<tr>
<td>2</td>
<td>--</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (246)</td>
<td>128-30</td>
<td>73</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (352)</td>
<td>218-220</td>
<td>89</td>
<td>0.51</td>
</tr>
<tr>
<td>4a</td>
<td>H</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (320)</td>
<td>270-72</td>
<td>76</td>
<td>0.85</td>
</tr>
<tr>
<td>4b</td>
<td>p-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (350)</td>
<td>263-65</td>
<td>79</td>
<td>0.79</td>
</tr>
<tr>
<td>4c</td>
<td>3,4,5-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (410)</td>
<td>266-68</td>
<td>82</td>
<td>0.83</td>
</tr>
<tr>
<td>4d</td>
<td>o-Cl</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (354)</td>
<td>252-54</td>
<td>65</td>
<td>0.65</td>
</tr>
<tr>
<td>4e</td>
<td>p-OH</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (336)</td>
<td>266-68</td>
<td>57</td>
<td>0.63</td>
</tr>
<tr>
<td>4f</td>
<td>o-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (336)</td>
<td>293-95</td>
<td>91</td>
<td>0.64</td>
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<tr>
<td>4g</td>
<td>p-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (334)</td>
<td>278-80</td>
<td>78</td>
<td>0.97</td>
</tr>
<tr>
<td>4h</td>
<td>p-N(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (363)</td>
<td>293-95</td>
<td>80</td>
<td>0.68</td>
</tr>
<tr>
<td>4i</td>
<td>o-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (365)</td>
<td>260-62</td>
<td>85</td>
<td>0.80</td>
</tr>
<tr>
<td>4j</td>
<td>p-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt; (365)</td>
<td>120-22</td>
<td>55</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Compounds were taken at concentrations of 100, 50 and 25 μg mL<sup>-1</sup> in DMF. <i>M. tuberculosis</i> H<sub>3</sub>Rv strain was used in Middle brook 7H-9 broth which was inoculated with standard as well test compounds and incubated at 37°C for 4 weeks. The bottles were inspected for growth twice a week for a period of three weeks. Readings were taken at the end of 4 weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. Compounds (4f, 4g, 4i and 4j) were showed good activity at concentration 25 μg mL<sup>-1</sup> when compared to reference drugs streptomycin (7.5 μg mL<sup>-1</sup>) and ciprofloxacin (10 μg mL<sup>-1</sup>). The results are shown in Table 2.

**Antibacterial Activity**

All the compounds were subjected to <i>in vitro</i> screening against Gram-negative <i>Escherichia coli</i> ATCC 25922 and <i>Klebsiella pneumonia</i> ATCC 29665 Gram-positive <i>Staphylococcus aureus</i> ATCC 12598 and <i>Enterococcus faecalis</i> ATCC 35550. The Minimum Inhibitory Concentration (MIC) was determined using tube dilution method according to standard procedure (Reimer et al., 1981). Muller-Hilton broth was used as culture medium. Sterilized medium was dispensed in each borosilicate glass test tubes. The drug solution was added in order to attain final concentration of 1500, 50, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1 μg mL<sup>-1</sup> in DMF. Inoculum's of standard suspension (0.1 mL of the test organism strain which contains 5×10<sup>5</sup> colony forming units mL<sup>-1</sup>) was added to tubes. The tubes were incubated at 37°C for 24 h and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point and Minimum Inhibitory Concentration (MIC) testing results are presented in Table 2.

**Antifungal Activity**

The test compounds were evaluated at 500, 250, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1 μg mL<sup>-1</sup> concentrations for their in vitro antifungal activity using Sabouraud’s Dextrose agar medium. Dimethyl formamide (DMF) was used as solvent for sample preparation. The Minimum Inhibitory
Table 2: Antimycobacterial, antibacterial and antifungal activities of the synthesized compounds (4a-6i) (MIC in μg mL⁻¹)

<table>
<thead>
<tr>
<th>Compound</th>
<th>M. tuberculosis H37Rv</th>
<th>K. pneumoniae</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>E. faecalis</th>
<th>C. albicans</th>
<th>A. fumigatus</th>
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<tbody>
<tr>
<td>4a</td>
<td>100.0</td>
<td>250.00</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>16.12</td>
<td>125.00</td>
<td>&gt;500</td>
</tr>
<tr>
<td>4b</td>
<td>100.0</td>
<td>125.00</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>8.00</td>
<td>62.50</td>
<td>&gt;500</td>
</tr>
<tr>
<td>4c</td>
<td>50.0</td>
<td>4.00</td>
<td>&gt;500</td>
<td>1.00</td>
<td>1.00</td>
<td>&gt;500</td>
<td>16.12</td>
</tr>
<tr>
<td>4d</td>
<td>50.0</td>
<td>2.00</td>
<td>&gt;500</td>
<td>8.00</td>
<td>16.12</td>
<td>&gt;500</td>
<td>&gt;500</td>
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<tr>
<td>4e</td>
<td>&gt;100.0</td>
<td>31.25</td>
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<td>31.25</td>
<td>&gt;500</td>
<td>8.00</td>
</tr>
<tr>
<td>4f</td>
<td>25.0</td>
<td>4.00</td>
<td>&gt;500</td>
<td>4.00</td>
<td>8.00</td>
<td>&gt;500</td>
<td>16.12</td>
</tr>
<tr>
<td>4g</td>
<td>25.0</td>
<td>2.00</td>
<td>&gt;500</td>
<td>31.25</td>
<td>16.12</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>4h</td>
<td>50.0</td>
<td>4.00</td>
<td>&gt;500</td>
<td>4.00</td>
<td>8.00</td>
<td>&gt;500</td>
<td>31.25</td>
</tr>
<tr>
<td>4i</td>
<td>25.0</td>
<td>4.00</td>
<td>&gt;500</td>
<td>31.25</td>
<td>31.25</td>
<td>&gt;500</td>
<td>4.00</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10.0</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Concentration (MIC) was determined and interpreted for *Candida albicans* ATCC 2091 and *Aspergillus fumigates* ATCC 13073 according to the procedure described by Lee et al. (2000). Sabouraud's Dextrose agar medium containing fluconazole as well as control Sabouraud's dextrose agar medium was inoculated with the microorganisms and incubated at 28°C for 48 h. The results of activities are presented in Table 2.

RESULTS AND DISCUSSION

In this study, we have synthesized a new series of Schiff's bases coupled with 3-methylquinolin-2(1H)-one. Yields of all synthesized compounds were in the range of 55-91%. All derivatives were evaluated for their Minimum Inhibitory Concentration (MIC) for the selected species of microorganisms. Compounds (4a-4i) were tested against *M. tuberculosis* H₃⁷Rv at three concentrations of 100, 50 and 25 μg mL⁻¹. Among them, compounds (4f, 4g, 4i and 4j) have shown good in vitro antimycobacterial activity (25 μg mL⁻¹) and compounds (4g, 4i) showed promising antibacterial and antifungal activity against laboratory isolates of *Staphylococcus aureus* ATCC 12598, *Enterococcus fecalis* ATCC 35550, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 29665, *Candida albicans* ATCC 2091 and *Aspergillus fumigates* ATCC 13073 and results are summarized in Table 2. All the evaluated compounds have shown considerable in vitro antimicrobial activity against tested strains of organisms except *E. coli* and *Candida albicans*. The results of antimicrobial testing revealed that the new derivatives showed broad spectrum of in vitro activity when compared to reference drug viz; streptomycin, ciprofloxacin and fluconazole.

Although, rarely described in the nature, synthetic quinoline ring is a part of a number of antibiotics such as echinomycin, leromycin and actinomycin, which are known to inhibit the growth of Gram-positive bacteria. The objectives of the project were to synthesize and evaluate the new derivatives bearing quinoline moiety. Initially, starting compound (1) 3-methylquinolin-2(1H)-one was prepared by condensation of o-phenylenediamine with pyruvic acid in aqueous medium, which on treatment with ethylchloroacetate in dimethylformamide (DMF) yielded ethyl 2-(3-methyl-2-oxoquinolin-1(2H)-yl)acetate (2). Compound (2) and hydrin hydrate were refluxed in ethanol medium to obtain 2-(3-methyl-2-oxoquinolin-1(2H)-yl)acetic acid hydrizide (3). A mixture of compound (3) and different aromatic aldehydes in DMF was refluxed to give N-((substituted phenyl)methylidene)-2-(3-methyl-2-oxoquinolin-1(2H)-yl)acetohydrazides (4a-4j). The compounds were confirmed by spectral studies such as IR, ¹H NMR and LCMS. Yields of all synthesized compounds were in the range of 55-91%. All the above reactions are briefly summarized in scheme Fig. 1. The infrared spectrum of intermediate (2) shows a sharp peak for C=O of ester at 1741 cm⁻¹. Conversion of ethyl ester into hydrizide was evidenced by appearance of sharp doublet at 3332 and 3209 cm⁻¹ due to NH₂. Similarly the signals of ¹H NMR spectrum at δ ppm 2.44 and δ ppm 9.37
confirm the NH$_2$ protons of hydrazide and NH of -CONH, respectively. The formation of Schiff base was evidenced by the disappearance of NH$_3$ protons and appearance of azomethine (-N = CH) proton peak at δ ppm 8.19. The multiplet signals at δ ppm 7.26-8.45 are characteristic aromatic ring protons. In all the compounds, a sharp singlet at δ ppm 2.35-2.50 is due to the protons of -CH$_3$ attached to the quinoxaline ring (Ali et al., 2000). Further compounds were confirmed by the LCMS and are in accordance with the proposed structures. In order to evaluate the efficacy as antimicrobial agents, compounds (4a-4j) were subjected to antimycobacterial, antibacterial and antifungal screening. Compounds (4f, 4g, 4i and 4j) have showed good antimycobacterial activity and compounds (4g and 4j) showed promising antibacterial and antifungal activity. From the biological studies it was possible to observe that some of the substituent on the phenyl ring of quinoxalimone hydrazones influenced the activity (Table 2).

In conclusion, the results of antimicrobial testing revealed the compounds to possess broad spectrum in vitro activity. Therefore, this study would be fruitful matrix for the development of novel class of antimicrobial agents. It is convincing that, derivatives showing significant antibacterial and antifungal activities can be further modified to exhibit better potency as that of standard drugs.

ACKNOWLEDGMENTS

Authors are thankful to Prof. B.G. Shivananda, Principal Al-Ameen College of Pharmacy, Bangalore for providing necessary facilities and Head of the department of Inorganic Chemistry, Indian
Institute of Science Bangalore and the director, Astra-Zeneca India Pvt. Limited for spectral analysis. Authors are also thankful to the HOD, M.M.'s N.G. Halgarker Institute of Dental Sciences and Research Center, Belgaum, India, for providing facilities for biological studies.

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