CHAPTER 4

SYNTHESIS, CHARACTERIZATION AND in vitro ANTIBACTERIAL AND ANTIFUNGAL EVALUATION OF NOVEL HALOPYRAZOLE DERIVATIVES


4.1. REVIEW AND LITERATURE

Research and development of potent and effective antimicrobial agents represent one of the most important advances in therapeutics, in the control of serious infections as well as in the prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery.\(^1\) In recent years, the number of life-threatening infectious diseases caused by multi-drug resistant Gram-positive and Gram negative pathogen bacteria have reached an alarming level in many countries around the world.\(^2\) \(^3\) Over the past decades, these enteric bacterial infections are responsible for morbidity and mortality in immuno-compromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant cases mostly in developing countries and areas such as the Indian sub-continent, part of South America, tropical part of Africa, etc.\(^4\)-\(^6\) Every year millions of people are being killed by some or the other Gram-positive and Gram-negative strains of bacteria. These bacteria mostly lead to food poisoning, rheumatic, salmonellosis and diarrhea.\(^7\) Thus, antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms.\(^8\) Furthermore, the pharmacological drugs available are either too expensive or have undesirable side effects or contraindications.\(^9\) A number of clinical reports in the United States and worldwide have independently
described the emergence of vancomycin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and other human pathogen Gram-negative isolates\(^\text{10}\). *Escherichia coli* are responsible for the world’s most common and serious infectious diseases like invasive dysentery and diarrhea, septicemia, inflammations of liver and gall bladder, appendix, meningitis, pneumonia etc.\(^\text{11-13}\) Generally, there are complains of abscess of the brain which is a very bad problem of *E. coli* infection.\(^\text{14}\) In case of *E. coli* infection, amoxicillin, norfloxacin and ciprofloxacin are generally used, but have harsh side effects.\(^\text{15}\) Toxicity and resistance to the drugs also play important role in the treatment failure.\(^\text{16}\) The different parasitic bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *E. coli* have important effects on the human’s mucosal health. The infections with these microorganisms may have significant impact on huge demolition of host tissue and severe diseases. More than 90 % of the cases of vaginitis are of candidiasis, trichomoniasis, and bacterial vaginosis\(^\text{17}\). Thus, these microorganisms commonly infect healthy people in large communities, thus creating a serious health problem around the globe. Therefore, the discovery of novel and potent antimicrobial agents is the best way to develop effective therapies. For this reason, the present stratagem for the synthesis of new compounds is aimed in the direction of developing new pyrazole derivatives to inhibit the growth of Gram-positive, Gram-negative bacteria, and fungi. Antibacterial and antifungal activities of the azoles are the most widely studied and some of them are in clinical practice as antimicrobial agents.\(^\text{18}\) In particular, pyrazoles gained much attention as antimicrobial agents after the discovery of natural pyrazole C-glycoside pyrazofurin which showed broad spectrum antimicrobial activity.\(^\text{19}\)
Pyrazoles have been studied for over a century as an important class of heterocyclic compounds and still continue to attract considerable attention, as they are the core structure of numerous biologically active compounds, including blockbuster drugs such as Celebrex an inhibitor of cyclooxygenase (COX-2) used as potent anti-inflammatory, \textsuperscript{20} Viagra, an inhibitor of 5-phosphodiesterase, used for the treatment of erectile dysfunction, \textsuperscript{21} Acomplia, antagonist of the CB-1 cannabinoid receptor, used for the treatment of obesity, \textsuperscript{22} Mepiprazole for the treatment of anxiety neuroses. \textsuperscript{23} Besides this, pyrazole derivatives are reported to have the broad spectrum of biological activities, such as anti-inflammatory\textsuperscript{24}, herbicidal\textsuperscript{25}, cytotoxic\textsuperscript{26}, analgesic\textsuperscript{27}, cholesterol-lowering\textsuperscript{28}, hypoglycemic\textsuperscript{29}, antihypertensive \textsuperscript{30}, antidepressant, anticonvulsant, \textsuperscript{31} antiviral, \textsuperscript{32} antiangiogenic activity \textsuperscript{33} and A3 adenosine receptor antagonists\textsuperscript{34} etc. They are also found applications as pharmaceuticals, agrochemicals, photographic, sunscreen materials etc.

Some of the pyrazole containing drugs having wide variety of activities are given below:
4.2. PRESENT WORK

A systematic investigation of this class of heterocyclic moiety revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead.\textsuperscript{35} Therefore, in view of the above-mentioned findings and to identify new candidates that may be of value in designing new, potent, selective and less toxic antimicrobial agents, this chapter describes the synthesis and antimicrobial evaluation of halopyrazole derivatives.

This chapter is divided into two sections.

Section A: Synthesis of novel halo pyrazole derivatives

In this section synthesis of two novel halopyrazoles (187 and 189) is described using 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (105) as starting material.

4.3. RESULTS AND DISCUSSION

5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde was obtained by Vilsmeier Haack reaction of pyrazolone.\textsuperscript{36} Due to the exceptional reactivity of formyl group in 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (105), it was taken as synthon for the generation of novel halopyrazoles. Thus, in an initial attempt to synthesize (187), (105) was heated with 5-acetyl-1,3-dimethylbarbituric (174d) acid in the presence of piperidine in ethanol followed by addition of bromine at room temperature (Scheme 69).
As expected, (187) was obtained in good yield. A plausible mechanism for the formation of (187) has been depicted in Scheme 70. As clear from Scheme 70, Claisen Schmidt condensation takes place between heteroaldehyde (105) and 5-acetyl 1,3-dimethyl barbituric acid (174d) in the presence of piperidine to give heterochalcone (186a) which reacts with bromine to form dibromodihydro derivative (186b). Subsequently the intermediate (186b) undergoes dehydrobromination to give final product (187). In order to synthesize a library of bromopyrazoles, similar reaction was carried out employing heteroaldehyde (105) and (188) under reflux conditions followed by addition of bromine at room temperature (Scheme 69). However, the reaction stopped at the stage of (189) and did not give bromopyrazole (190) after addition of bromine. The failure of reaction of (189) with bromine to give expected dibromo dehydro derivatives is may be due to the steric hindrance from bulky aromatic and heteroaromatic ring (Scheme 69).
The structures of the compounds isolated were characterized by elemental and spectral analysis (IR, $^1$H NMR, $^{13}$C NMR and Mass spectrometry). The IR spectrum (Fig. 24) of (187) showed the carbonyl absorption band of barbituric acidity moiety at 1730 cm$^{-1}$. The absorption bands for carbonyl group and carbon–carbon double bond of the α, β-unsaturated system appeared at 1689 and 1623 cm$^{-1}$, respectively. The $^1$H NMR spectrum (Fig. 25) showed $H_a$ proton as sharp upfield singlet at δ 7.81. The aromatic protons of the N-phenyl-pyrazole-moiet were present in the form of multiplet at δ 7.46–7.62. Six protons of N–CH$_3$ groups were discernible as two sharp singlets at δ 3.32 and 3.48 whereas protons of CH$_3$ group of pyrazole unit were present in the form of another sharp
singlet at δ 2.61. Further confirmation of the structure was done by mass spectrum (Fig. 26), which showed M⁺ at 480.6 as base peak. The ¹³C NMR spectrum (Fig. 27) was also in accordance with the proposed structure given in the experimental section. The IR spectrum (Fig. 28) of (189) showed two strong absorption band at 1597 and 1535 cm⁻¹ for C=C and C=N bonds respectively. The ¹H NMR (Fig. 29) exhibited a sharp singlet at δ 2.54 for three methyl protons of pyrazole moiety. Five aromatic protons of pyrazole unit appeared as multiplet in the region δ 7.35-7.57. The protons Hₐ and Hₗ were present as trans coupled doublets at δ 7.20 (J = 16.5 Hz) and 7.66 (J = 16.5 Hz). Further confirmation of the structure was provided by mass spectrometry (Fig. 30) which showed M⁺ at 384.12 as base peak. The ¹³C NMR spectrum (Fig. 31) was also in accordance with the proposed structure. The other peaks were observed at their normal position and are given in the experimental section.
4.4. EXPERIMENTAL

Melting points were taken in Reichert Thermover instrument and are uncorrected. The IR spectra were recorded on Perkin Elmer RXI spectrometer in KBr. $^1$H NMR spectra were recorded on Bruker DRX-300 and Bruker Avance II-400 spectrometer using tetramethyl silane (TMS) as an internal standard. $^{13}$C NMR spectra were recorded on a Bruker DRX400 Spectrometer (100 MHz) with DMSO. Mass spectra were recorded on JEOL-Accu TOF JMS-T100LC DART-MS spectrometer. Microanalytical data were collected using Carlo Erba analyzer model 1108. The purity of all compounds was checked by TLC on glass plates (20 ×5 cm) coated with silica gel (E-Merck G254, 0.5 mm thickness). The plates were run in chloroform–methanol (4:1 V/V) mixture and were visualized by iodine vapors. The 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde $^{36}$ and 5-acetyl-1,3-
dimethylbarbituric acid were synthesized from 3-methyl-1-phenylpyrazole-5-one and 1,3-dimethylbarbituric acid, respectively by reported procedures.

4.4.1. General Procedure for the synthesis of compound 187

To a well stirred solution of 5-acetyl-1,3-dimethylbarbituric acid (174d) (4.52 mmol) in ethanol (12 mL) containing pyridine (0.5 mL), 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (105) (4.52 mmol) was added in the portion. The reaction mixture was then stirred at room temperature for 1 h. Bromine (4.52 mmol) was then added dropwise to the vigorously stirred solution over a period of 20 min. After complete addition of Br₂ the reaction mixture was further stirred for another 3 h. The monobromoderivative (187) was precipitated, filtered off, and washed with 15 mL of ether to remove the excess of bromine. Further purification was made by recrystallization from the chloroform–methanol (4:1 V/V) mixture.

4.4.2. General Procedure for the synthesis of compound 189

A mixture of 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (105) (4.52 mmol), 2,4-dinitrotoluene (188) (4.52 mmol) and pyridine (0.3 mL) in ethanol (12 mL) was refluxed in a heating mantle for about 2.5 h. On completion of reaction (as checked by TLC), the reaction mixture was poured into 25 mL ice cold water, acidified with conc-HCl. The solid, thus, obtained was filtered washed with water, methanol, dried and purified by recrystallization from chloroform–methanol (3:2 V/V) mixture.
4.5. Spectral Data

(Z)-2-Bromo-3-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-1-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)prop-2-ene-1-one (187)

Purification was made by recrystallization from the chloroform–methanol (4:1 V/V) mixture.

Pale yellow crystals.

M.p. : 180–182 °C.

IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$ : 1730 (C=O), 1689 (C=O), 1623 (C=C).

$^1$H NMR (400 MHz, CDCl$_3$) : $\delta$ 2.61 (s, 3H, CH$_3$), 3.32 (s, 3H, N–CH$_3$), 3.48 (s, 3H, N–CH$_3$), 7.46–7.62 (m, 5H, Ar–H), 7.81 (s, 1H, H$_a$).

$^{13}$C NMR (100 MHz, DMSO) : $\delta$ 14.27, 27.73, 29.28, 38.94, 108.52, 124.53, 124.99, 128.40, 128.67, 128.79, 137.13, 148.57, 157.13, 165.12, 174.42.

ESI–MS : $m/z$ 480.6 (M$^+$).

Elemental analysis

For C$_{19}$H$_{16}$N$_4$O$_4$BrCl : Calculated C, 47.57; H, 3.35; N, 11.67.

Found: C, 47.42; H, 3.49; N, 11.56
**5-Chloro-4-[2-(2,4-dinitrophenyl)-vinyl]-3-methyl-1-phenyl-1H-pyrazole (189)**

Purification was made by recrystallization from the chloroform–methanol (3:2 V/V) mixture.

Yellow crystals.

**M.p.** : 122–125 °C.

**IR (KBr) $\nu_{max}$/cm$^{-1}$** : 1597 (C=C), 1535 (C=N), 1345 (C–N).

**$^1$H NMR (300 MHz, CDCl$_3$)** : $\delta$ 2.54 (s, 3H, CH$_3$), 7.20 (d, 1H, J= 16.5 Hz, H$_a$), 7.35–7.57 (m, 5H, Ar–H), 7.66 (d, 1H, J= 16.5 Hz, H$_b$), 8.00 (d, 1H, J= 8.7 Hz, H$_c$), 8.45 (d, 1H, J= 8.7 Hz, H$_d$), 8.85 (s, 1H, H$_e$).

**$^{13}$C NMR (100 MHz, DMSO)** : $\delta$ 14.65, 115.11, 117.54, 120.84, 120.99, 124.98, 125.28, 128.72, 129.28, 129.39, 139.37, 147.12, 149.05, 151.85.

**ESI–MS** : $m/z$ 384.12 (M$^+$).

**Elemental analysis**

For C$_{18}$H$_{13}$N$_4$O$_4$Cl : Calculated C, 56.19; H, 3.40; N, 14.56.

Found: C, 56.06; H, 3.53; N, 14.51
Fig. 24. IR spectrum of 187

Fig. 25. $^1$H NMR spectrum of 187
Fig. 26. Mass spectrum of 187

Fig. 27. $^{13}$C NMR spectrum of 187
Fig. 28. IR spectrum of 189

Fig. 29. $^1$H NMR spectrum of 189
Fig. 30. Mass spectrum of 189

Fig. 31. $^{13}$C NMR spectrum of 189
Section B: Antimicrobial evaluation of pyrazole derivatives

The synthesized compounds described in the section A have been screened for their in vitro antibacterial and antifungal activities.

4.6. In vitro antibacterial studies of compounds 187 and 189

4.6.1. Microorganism used

The newly synthesized compounds were screened for their antibacterial activity against *Streptococcus pyogenes* (clinical isolate), Methicillin resistant *Staphylococcus aureus* (MRSA+ve), *Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneumoniae* (clinical isolate) and *Escherichia coli* (ATCC-25922) bacterial strains by disk diffusion method. A standard inoculums (1-2 X 10^7 c.f.u./ml 0.5 Mc Farland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Ciprofloxacin (30 µg) was used as positive control while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 16.
### Table 16 Antibacterial activity of compounds 187, 189 and positive control ciprofloxacin.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>S. pyogenes</strong></td>
<td><strong>MRSA</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>187</td>
<td>19.4±0.4</td>
<td>19.4±0.4</td>
</tr>
<tr>
<td>189</td>
<td>19.2±0.2</td>
<td>18.2±0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>22.5±0.4</td>
<td>21.5±0.4</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (Standard); ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).<sup>a</sup> Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 5 X 10<sup>5</sup> c.f.u./mL of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bactericidal concentration (MBC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 17.
Table 17 MIC and MBC results of compounds 187, 189 and positive control ciprofloxacin.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>MRSA</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>187</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>189</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

MIC (µg/mL), minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/mL), minimum bactericidal concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

4.7. In vitro antifungal studies of compounds 187 and 189

4.7.1. Microorganism used

For assaying antifungal activity Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes and Penicillium marneffei were recultured in DMSO by agar diffusion method.39

Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The fungal activity of each compound was compared with griseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 18.
Table 18 Antifungal activity of compounds (187, 189). Positive control (Griseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm). Diameter of zone of inhibition (mm).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>187</td>
<td>21.4±0.2</td>
<td>19.9±0.3</td>
<td>16.2±0.2</td>
<td>13.5±0.4</td>
</tr>
<tr>
<td>189</td>
<td>21.2±0.4</td>
<td>18.4±0.2</td>
<td>15.3±0.4</td>
<td>12.2±0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>30.5±0.2</td>
<td>26.5±0.2</td>
<td>23.5±0.3</td>
<td>21.5±0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

CA; Candida albicans, AF; Aspergillus fumigatus, TM; Trichophyton mentagrophytes, PM; Penicillium marneffei.

The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4$– $6 \times 10^4$ c.f.u./mL. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungi was regarded as minimum inhibitory concentration (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 19.
Table 19. MIC and MFC of compounds 187, 189. Positive control griseofulvin.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>187</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>100</td>
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<tr>
<td>189</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

CA; Candida albicans, AF; Aspergillus fumigatus, TM; Trichophyton mentagrophytes, PM; Penicillium marneffei. MIC (µg/mL), minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of fungi completely; MFC (µg/mL), minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungi completely.

The PBE (percentual bacteriostatic efficiency, %) was obtained as, \( PBE = \frac{100}{MIC} \) and fungicidal/fungistatic activity (MFC/MIC) as obtained are presented in Table 20. The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC ≤ 4) activity and the results have been summarized in Table 20.

Table 20 PBE and fungicidal/fungistatic activity (MFC/MIC) of compounds 187 and 189.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>PBE=100/MIC</th>
<th>MFC/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria tested</td>
<td>Fungi tested</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>MRSA</td>
</tr>
<tr>
<td>187</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>189</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SP; S. pyogenes, MRSA; Methicillin resistant Staphylococcus aureus, PA; P. aeruginosa, KP; K. pneumoniae, EC; E. coli, CA; C albicans, AF; A. fumigatus, TM; T. mentagrophytes, PM; P. marneffei.
The investigation of antibacterial screening data (Tables 16 and 17) revealed that all the tested compounds (187) and (189) showed moderate to good bacterial inhibition. All the compound showed good inhibition against S. pyogenes, Methicillin resistant Staphylococcus aureus (MRSA +ve), P. aeruginosa, K. pneumonia and E. coli species. MIC of compounds was in the range of 12.5–50 µg/mL. The MBC of compounds was found to be two or four folds higher than the corresponding MIC results.

The antifungal screening data (Tables 18 and 19) showed moderate to good activity. The compounds (187 and 189) showed good fungicidal activity against C. albicans, A. fumigates, T. mentagrophytes and P. marneffei fungal strains. MIC of compounds was in the range of 12.5–50 µg/mL. The MBC of the compounds was found to be two or four folds higher than the corresponding MIC results. Most of the compounds showed good fungicidal activity against various fungal strains (Table 20).

4.8. CONCLUSION

In summary, a convenient method for the synthesis of novel halopyrazole derivatives has been developed. The procedure offers advantages such as mild reaction conditions as well as simple experimental and product isolation procedures, thus, making the current protocol as useful and interesting methodology for the synthesis of novel halopyrazoles in good yields from cheap and readily available starting materials. The antibacterial, antifungal screening data revealed that the halopyrazoles may be used as template for future development through modification and derivatization to design more potent and selective antimicrobial agents.
4.9. REFERENCES


