An Efficient Synthesis and Biological Properties of 2- [ (5-Methoxy-1H- Benzimidazol-2-yl) Sulfanyl] - N-Phenylacetamide Motief

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Abstract: A new series of 2- [ (5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-phenylacetamide have been synthesized by the condensation 2-mercapto-5-methoxybenzemidazole and 2- chloro – N – (Aryl) – acetamides. The novel compounds structure has been established on the basis of their substituted N-chloro aryl acetamide derivatives. All the compounds were characterized by Mass, FT-IR, CMR, ¹H-NMR spectroscopy as well as elemental analysis. These new compounds were evaluated for their in vitro antibacterial activity and anti fungal activity.

Keywords: Acetamide, anti fungal activity, bacterial activity and spectroscopy.

1. Introduction

The rapidly expanding population of immune compromised patient results in a corresponding increase of diseases caused by bacteria, fungi and other yeast. Infection caused by these microorganisms pose a serious challenge to the medical community and highlight the importance and urgent need for new, more potent and selective non-traditional antimicrobial agent. The incidence of bacterial infections has increased dramatically in recent years¹. The widespread use of antibacterial and antifungal drugs and their resistance against bacterial and fungal infections has led to serious health hazards. The resistance of wide spectrum antibacterial agents has prompted discovery and modification towards new antifungal and antibacterial drugs ²,³ In continuation of our interest on chemistry of functionalized chloroacetamide derivatives⁴, Because of the high mobility of chlorine atom and reactive N-H group, compounds containing chloroacetamide moiety are known to be useful synthetic scaffolds for design of aziridines, lactams, piperazines, oxazolidines, imidazolidines and tetrahydropyrimidines – precursors of heterocyclic carbones, macrocyclic ligands, dendrimers. ²-Chloracetamide derivatives found application in solid-state chemistry, in synthesis of aminoacids, natural compounds and their homologs, pharmacologically promising substances ¹⁴ and biomarkers, reagents for polymer modification, ion-exchange resins for heavy and radioactive metal sorption. Chloroacetamide pesticides and dyes are also well known. Thus, investigation of 2-chloroacetamide chemistry is an actual task both from theoretical and applied viewpoints.

2. Results and Discussion

2- [ (5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-phenylacetamide were obtained in 66-83% yield by converting 4-Methoxyacetanilide to the 4-Methoxy-2-nitroacetanilide (1) and 4-Methoxy-2-nitroaniline (2) by reaction with hydrochloric acid and alkaline NaOH, respectively. 4-Methoxy-2-nitroaniline was converted to 4-Methoxy-1,2-phenylene diamine (3) . 4-Methoxy-1,2-phenylene diamine reacted with CS₂ and ethanol was converted to 2-mercapto-5-methoxybenzemidazole (4) . Reaction between 2-mercapto-5-methoxybenzemidazole (4) with 2-chloro-N- (aryl) -acetamides (5) give compounds (6a-j) .

Mass spectral data support the proposed structures. The mass spectrum showed various characteristic peaks. A peak at m/z 327 was assigned to the molecular ion.

The FTIR spectrum showed absorption bands at 675 cm⁻¹ (C=O stretching in amide) , 3298 cm⁻¹ (NH- stretching in amide) , 1654 cm⁻¹ (C=C stretching in amide) 1595 cm⁻¹ (S=C=O stretching in thioether linkage) 1396 cm⁻¹ (C-CH₃ stretching in aromatic ring) respectively. 2835 cm⁻¹ (C-H stretching in methylene) , 1271 cm⁻¹ (C-O aryl stretching) . 1168 cm⁻¹ (C-O alkyl stretching).

The ¹H-NMR spectrum of (6a-j) showed characteristic signals at 6.73 to 7.60 ppm which were assigned to the aromatic protons. A signal at 4.18 ppm was assigned to the methylene proton. A signal at 2.49 ppm was assigned to the methyl proton. The singlet 3.75 ppm was assigned to the methoxy protons, respectively.

The 13C-NMR spectrum of (6a-j) showed characteristic peaks at 104.2-153.9 ppm which were assigned to the aromatic carbon atoms. A peak at 165.08 ppm was attributed to the carbonyl carbon atom.. The peaks at 56.5 ppm were assigned to methoxy carbon atoms. A peak at 20.89 ppm was assigned to the methoxy carbon atoms.

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3. Experimental

All the melting points were recorded on Cintex melting point apparatus and are uncorrected. IR spectra in KBr were recorded on Schimadzu FTIR spectrophotometer in cm⁻¹. 1HNMR spectra were recorded in CDC13 or DMSO on a Bruker DRX-400 MHZ NMR instrument. Chemical shifts were reported in ppm using TMS as internal standard on δ scale. Mass spectra of compounds were recorded on mass spectrometer (Agilent 1100 series). Completion of the reactions was monitored time to time by TLC using E-Merck 0.25 mm silica gel plates and water: ethanol (4:6) as solvent system.

Step – 1

(i) Synthesis of 4-Methoxyacetanilide

P- Anisidine (0.04 mole) was stirred rapidly with glacial acetic acid (12 ml). Acetic anhydride (0.04 ml) was added to it. All at once. Acetylated product, 4-Methoxyacetanilide was formed within a short time period. The crude product was recrystallized from a mixture of water-ethanol (4:6) to obtain pure compound in 70% yield

(ii) Synthesis of 4-Methoxy-2-nitroacetanilide

4-Methoxyacetanilide was nitrated at 2-position using a mixture of conc. Sulfuric acid (6.0 ml) and conc. Nitric acid (2.0 ml). The nitrating mixture was cooled to 0 C and 4-Methoxyacetanilide was added to it at such a rate that temperature did not exceed 5 C. After the addition, the reaction mixture was poured in ice-water. The precipitated yellow solid was washed with water and recrystallized from a mixture of water-ethanol (4:6) to obtain product in 86% yield.

(iii) Synthesis of 4-Methoxy-2-nitroaniline

4-Methoxy-2-nitroanilide was hydrolysed with hydrochloric acid (12.0 ml) for 30 min at 100 C. The reaction mixture was poured in ice-water. The precipitated mixture was filtered and washed with cold water. Recrystallized product was obtained pure compound in 70% yield.

(iv) Synthesis of 4-Methoxy-1,2-phenylenediamine

4-Methoxy-2-nitroanilide was reacted with methanolic ammonia (20%) at 20 C in a closed vessel. After heating for a few hours at room temperature, the solution was partially neutralized to get pH 5. Stannous chloride was removed by precipitating tin with H2S as tin sulfide and then filtering it. The filtrate was concentrated to a small volume, made strongly basic and then extracted with ether. Removal of ether gave a dark coloured base. Which was recrystallized from ethanol.

(V) Synthesis of 2-mercapto-5-methoxybenzimazole

Potassium hydroxide (0.03 mol) was dissolved in a mixture of ethanol (30.0 ml) and water (30.0 ml). To this Cs2 (0.03 ml) was added with stirring. This mixture was boiled and then the solution of 4-Methoxy-1,2-phenylenediamine (0.03 ml) in ethanol (20.0 ml) was added drop wise to it. After refluxing the reaction mixture for 6 hr. ethanol was removed. The white residue obtained was dissolved in water and the product was precipitated by the addition of dilute acetic acid (50%). It was then recrystallized from a mixture of water-ethanol (1:1).


In benzene (30.0 ml), chloroacetyl chloride (0.02 mole) and 4-6 drops of triethyl amine was added, the mixture was stirred in ice bath. The solution of aryl amine (0.02 mole) in benzene (30.0 ml) was added drop-wise and refluxed for 4 hours and the reaction mixture was cooled to ambient temp. The resulting ppt. were filtered and washed with benzene purified by re- crystallization from alcohol.

Step – 3 Synthesis of 2- [ (5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-phenylacetamide

A mixture of 2-chloro-N- (aryl) -acetamide (0.01M) , 2-mercapto-5-methoxybenzimidazole (0.01M) in 30ml dry acetic anhydride and anhydrous K2CO3 (0.02M) was stirred for 3 hour at room temperature and poured into ice. The product was filtered and washed with cold water. Recrystallized from ethanol, The Progress of reaction was monitored by TLC using Toluen: acetone (8: 2) as Eluent. Purificaton of all the synthesized compounds was achieved by recrystallization and purity of each compound was monitored by thin layer TLC.
FTIR (KBr, cm$^{-1}$): 3320 (N-H), 1278 (C=O aroyl stretching), 1157 (C-O alkyl stretching) 1662 (C=O in amide), 2846 (C-H in methylene). 1612 (S-C=O in thioether linkage), 1043 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 3.79 (s, 3H, OCH$_3$), 4.37 (s, 2H, CH$_2$). 6.89 (dd, J=9.7, 2H, Ar-H), 7.29 (dd, J=7.9, 2H, Ar-H), 6.87 (dd, J=8.5, 1H, Ar-H), 7.73 (dd, J=9.4, 1H, Ar-H), 7.34 (dd, J=10.5, 1H, Ar-H), 10.57 (s, 1H, Ar-H), 12.37 (s, 1H, NH$_2$), 137.8, 118.8, 130.9, 122.7 (Ar-C), 162.8 (C=O), MS (m/z): 347 (M$^+$).

N-phenyl-2-[5- (3,4,5-trimethoxyphenyl) -1,3,4-oxadiazol-2-yl] sulfanyl]acetamide (5i)

FTIR (KBr, cm$^{-1}$): 3310 (N-H), 1281 (C=O aroyl stretching), 1155 (C-O alkyl stretching) 1652 (C=O in amide), 2819 (C-H in methylene). 1576 (S-C=O in thioether linkage), 1413 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 3.64 (s, 3H, OCH$_3$), 4.37 (s, 2H, CH$_2$), 7.19 (m, 1H, Ar-H), 7.28 (m, 1H, Ar-H), 7.09 (m, 1H, Ar-H), 6.73 (m, 1H, Ar-H), 6.97 (dd, J=8.3, 1H, Ar-H), 7.75 (dd, J=9.6, 1H, Ar-H), 7.57 (s, 1H, Ar-H), 10.57 (s, 1H, Ar-H), 12.37 (s, 1H, NH$_2$), 137.5, 113.2, 141.7, 137.1, 150.0 (Ar-C), 175.00 (C=O), MS (m/z): 347 (M$^+$).

N- (3-methoxyphenyl)-2-[5- (3,4,5-trimethoxyphenyl) -1,3,4-oxadiazol-2-yl] sulfanyl]acetamide (5j)

FTIR (KBr, cm$^{-1}$): 3312 (N-H), 1287 (C=O aroyl stretching), 1137 (C-O alkyl stretching) 1672 (C=O in amide), 2856 (C-H in methylene). 1633 (S-C=O in thioether linkage), 1419 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 3.83 (s, 3H, OCH$_3$), 4.09 (s, 2H, CH$_2$), 6.90 (dd, J=8.2, 2H, Ar-H), 7.37 (dd, J=8.5, 2H, Ar-H), 6.93 (dd, J=7.8, 1H, Ar-H), 7.81 (dd, J=9.8, 1H, Ar-H), 10.22 (s, 1H, NH$_2$), 112.4 (s, 1H, NH$_2$), 137.5, 113.2, 141.7, 137.1, 150.0 (Ar-C), 175.00 (C=O), MS (m/z): 347 (M$^+$).

N- (3-chlorophenyl)-2-[5- (3,4,5-trimethoxyphenyl) -1,3,4-oxadiazol-2-yl] sulfanyl]acetamide (5f)

FTIR (KBr, cm$^{-1}$): 3289 (N-H), 1278 (C=O aroyl stretching), 1158 (C-O alkyl stretching) 1664 (C=O in amide), 2845 (C-H in methylene). 1607 (S-C=O in thioether linkage), 1397 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 3.67 (s, 3H, OCH$_3$), 4.05 (s, 2H, CH$_2$), 6.95 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.01 (m, 1H, Ar-H), 7.57 (m, 1H, Ar-H), 6.84 (dd, J=7.0, 1H, Ar-H), 7.33 (dd, J=7.0, 1H, Ar-H), 7.12 (s, 1H, Ar-H) 10.39 (s, 1H, NH$_2$), 12.67 (s, 1H, NH$_2$), 137.5, 113.2, 141.7, 137.1, 150.0 (Ar-C), 168.6 (C=O), MS (m/z): 347 (M$^+$).

N- (3-methylphenyl)-2-[5- (3,4,5-trimethoxyphenyl) -1,3,4-oxadiazol-2-yl] sulfanyl]acetamide (5g)

FTIR (KBr, cm$^{-1}$): 3313 (N-H), 1249 (C=O aroyl stretching), 1171 (C-O alkyl stretching) 1686 (C=O in amide), 2829 (C-H in methylene). 1617 (S-C=O in thioether linkage), 1410 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 2.39 (s, 3H, CH$_3$), 3.85 (s, 3H, OCH$_3$), 4.26 (s, 2H, CH$_2$), 7.13 (s, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.19 (m, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 6.93 (dd, J=8.0, 1H, Ar-H), 7.84 (dd, J=8.0, 1H, Ar-H), 7.13 (s, 1H, Ar-H) 10.47 (s, 1H, NH$_2$), 12.57 (s, 1H, NH$_2$), 137.5, 113.2, 141.7, 137.1, 150.0 (Ar-C), 168.6 (C=O), MS (m/z): 327 (M$^+$).

N- (2-chlorophenyl)-2-[5- (3,4,5-trimethoxyphenyl) -1,3,4-oxadiazol-2-yl] sulfanyl]acetamide (5h)

FTIR (KBr, cm$^{-1}$): 3327 (N-H), 1296 (C=O aroyl stretching), 1142 (C-O alkyl stretching) 1663 (C=O in amide), 2812 (C-H in methylene), 1617 (S-C=O in thioether linkage), 1389 (C-CH$_3$ in aromatic ring), $^1$H-NMR (DMSO-d$_6$, ppm): 3.89 (s, 3H, OCH$_3$), 4.03 (s, 2H, CH$_2$), 7.17 (m, 1H, Ar-H), 7.27 (m, 1H, Ar-H), 7.05 (m, 1H, Ar-H), 7.39 (m, 1H, Ar-H), 6.83 (dd, J=7.4, 1H, Ar-H), 7.73 (dd, J=8.1, 1H, Ar-H), 7.23 (s, 1H, Ar-H) 10.57 (s, 1H, NH$_2$), 12.46 (s, 1H, NH$_2$), 137.5, 113.2, 141.7, 137.1, 150.0 (Ar-C), 162.9 (C=O), MS (m/z): 356 (M$^+$).

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4. Reaction Scheme:

**Step -I**

\[
\begin{align*}
  &\text{Step -I} \\
  &\text{NHCOCH}_3 \xrightarrow{\text{HNO}_3, \text{H}_2\text{SO}_4} \text{NHCOCH}_3 \xrightarrow{\text{Alkali}} \text{NH}_2 \xrightarrow{\text{NO}_2, \text{Na}_2\text{S}} \text{NH}_2 \xrightarrow{\text{H}_3\text{CO}} \text{SH} \\
  &\text{(1)} \quad \text{(2)} \quad \text{(3)} \quad \text{(4)}
\end{align*}
\]

**Step -II**

\[
\begin{align*}
  &\text{NH}_2 + \text{R} + \text{Cl} + \text{Cl} \xrightarrow{\text{TEA / Benzene}} \text{R} \\
  &\text{(5)}
\end{align*}
\]

**Step -III**

\[
\begin{align*}
  &\text{H}_3\text{CO} \xrightarrow{\text{Dry Acetone / R.T}} \text{NH}_2 \xrightarrow{\text{Anhydrous K}_2\text{CO}_3} \text{R} \\
  &\text{(4)} \quad \text{(5)} \quad \text{(6)}
\end{align*}
\]

Where: R =

\[
\begin{align*}
  &\text{(a) H}_3\text{N} \quad \text{(b) H}_2\text{N} \quad \text{(c) H}_2\text{N} \quad \text{(d) H}_2\text{N} \quad \text{(e) NH}_2 \\
  &\text{(f) H}_2\text{N} \quad \text{(g) H}_2\text{N} \quad \text{(h) H}_2\text{N} \quad \text{(i) H}_2\text{N} \quad \text{(j) H}_2\text{N}
\end{align*}
\]

**Figure 1**

2-[(5-methoxy-1H-benzimidazol-2-yl)sulfanyl]-N-phenylacetamide (6 (a-j))
Table 1: Physical data of 2-[(5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-Phenylacetamide

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>% Yield (G.C.)</th>
<th>M.P. (°C)</th>
<th>Cal. %</th>
<th>Found %</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>C$<em>{10}$H$</em>{13}$N$<em>{2}$O$</em>{5}$S</td>
<td>327.4</td>
<td>80</td>
<td>148-150</td>
<td>62.36</td>
<td>62.40</td>
<td>5.23</td>
<td>5.27</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>C$<em>{10}$H$</em>{12}$FN$<em>{2}$O$</em>{5}$S</td>
<td>331.3</td>
<td>71</td>
<td>175-177</td>
<td>57.99</td>
<td>58.13</td>
<td>4.26</td>
<td>4.31</td>
</tr>
<tr>
<td>3</td>
<td>6c</td>
<td>C$<em>{13}$H$</em>{13}$ClFN$<em>{2}$O$</em>{5}$S</td>
<td>365.8</td>
<td>74</td>
<td>166-168</td>
<td>52.53</td>
<td>52.57</td>
<td>3.94</td>
<td>3.98</td>
</tr>
<tr>
<td>4</td>
<td>6d</td>
<td>C$<em>{13}$H$</em>{14}$N$<em>{2}$O$</em>{5}$S</td>
<td>358.3</td>
<td>71</td>
<td>230-233</td>
<td>53.62</td>
<td>53.67</td>
<td>4.44</td>
<td>4.48</td>
</tr>
<tr>
<td>5</td>
<td>6e</td>
<td>C$<em>{13}$H$</em>{13}$N$<em>{2}$O$</em>{5}$S</td>
<td>343.0</td>
<td>67</td>
<td>188-191</td>
<td>59.46</td>
<td>59.49</td>
<td>4.99</td>
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<tr>
<td>6</td>
<td>6f</td>
<td>C$<em>{14}$H$</em>{14}$ClN$<em>{2}$O$</em>{5}$S</td>
<td>347.8</td>
<td>63</td>
<td>162-164</td>
<td>55.25</td>
<td>55.29</td>
<td>4.06</td>
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<td>7</td>
<td>6g</td>
<td>C$<em>{13}$H$</em>{14}$N$<em>{2}$O$</em>{5}$S</td>
<td>327.4</td>
<td>71</td>
<td>198-200</td>
<td>62.36</td>
<td>63.40</td>
<td>5.23</td>
<td>5.27</td>
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<td>6h</td>
<td>C$<em>{15}$H$</em>{23}$N$<em>{2}$O$</em>{5}$S</td>
<td>356.4</td>
<td>60</td>
<td>230-233</td>
<td>60.65</td>
<td>60.69</td>
<td>5.66</td>
<td>5.71</td>
</tr>
<tr>
<td>9</td>
<td>6i</td>
<td>C$<em>{15}$H$</em>{24}$ClN$<em>{2}$O$</em>{5}$S</td>
<td>347.8</td>
<td>81</td>
<td>184-186</td>
<td>55.25</td>
<td>55.29</td>
<td>4.06</td>
<td>4.11</td>
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<td>10</td>
<td>6j</td>
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<td>392.2</td>
<td>63</td>
<td>210-212</td>
<td>48.99</td>
<td>49.03</td>
<td>3.60</td>
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</table>

Antibacterial Activity

This part deals with the in-vitro screening of newly prepared compounds for antibacterial activity. The species *S. aureus*, *E. coli*, *S. typhi* and *B. subtilis* have been taken for the antibacterial activities. Agar-cup method was carried out for the in-vitro screening for antibacterial activity. The results of the compounds synthesized given for antibacterial screening are mentioned in following Table.

Table 2: Antibacterial activity of standard drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(microgram/ml)</td>
<td>MTCC 443</td>
<td>MTCC 1688</td>
<td>MTCC 96</td>
<td>MTCC 442</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0.05</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>100</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<td>Ciprofloxacin</td>
<td>25</td>
<td>25</td>
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</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3: Antibacterial activity of 2-[(5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-Phenylacetamide

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Code No.</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>125</td>
<td>125</td>
<td>500</td>
<td>1000</td>
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<td>2</td>
<td>6b</td>
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<td>500</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>6c</td>
<td>125</td>
<td>125</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>4</td>
<td>6d</td>
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<td>500</td>
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<td>6i</td>
<td>500</td>
<td>250</td>
<td>250</td>
<td>250</td>
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<tr>
<td>10</td>
<td>6j</td>
<td>500</td>
<td>200</td>
<td>200</td>
<td>100</td>
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</tbody>
</table>

Antifungal Activity

This part deals with the in-vitro screening of newly prepared compounds for antifungal activity. The species *C. albicans, A. niger, A. clavatus* have been taken for the antifungal activities. Agar-cup method was carried out for the in-vitro screening for antifungal activity. The results of the compounds synthesized given for antifungal screening are mentioned in following table.

Table 4: Antifungal activity of standard drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>C. albicans</th>
<th>A. niger</th>
<th>A. clavatus</th>
</tr>
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<tbody>
<tr>
<td>(microgram/ml)</td>
<td>MTCC 227</td>
<td>MTCC 282</td>
<td>MTCC 1323</td>
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<tr>
<td>Nystatin</td>
<td>100</td>
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<td>100</td>
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Table 5: Antifungal activity of 2-[(5-methoxy-1H-benzimidazol-2-yl) sulfanyl] -N-Phenylacetamide

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<th>C. albicans</th>
<th>A. niger</th>
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5. Acknowledgement

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References

(b) Concannon, J.; Rodriguez-Solla, H.; Amo, V.;


