CHAPTER-2
RESULTS & DISCUSSION
SECTION-2.1
Synthesis and Cytotoxic Evaluation of 2-Aminoimidazole-Quinoline Hybrids Against Cancer and Primary Endothelial Cells
Synthesis and Cytotoxic Evaluation of 2-Aminoimidazole-Quinoline Hybrids Against Cancer and Primary Endothelial Cells

Cancer, a life threatening disease is now affecting the people at all ages and is responsible for increase in the mortality rate globally [1-3] In spite of availability of a large number of existing anti-cancer drugs, the development of new chemotherapeutics have always been one of the most noteworthy challenges due to non-selectivity and emergence of resistance by cancerous cells towards existing anti-cancer compounds. Therefore, a constant need to develop better alternatives to face such incoming problems in future is always in demand. There are a large number of heterocyclic compounds that have already been reported to exhibit anticancer properties [4].

Literature survey revealed that among heterocycles, imidazole derivatives have been proven as an excellent class of broad spectrum anti-cancer agents against a variety of cancer cell lines such as hepatocellular carcinoma, breast cancer, acute myelogenous leukemia, non-small cell lung carcinoma, etc. [5-9]. In past decades, 2-aminoimidazoles have gained much attention because of their high selectively as anti-cancer agents against some cancerous cells [10-12]. The dacarbazine (Figure 32), an antineoplastic drug bearing 2-aminoimidazole nucleus, has been utilized in the treatment of a variety of cancers such as malignant melanoma, Hodgkin lymphoma, sarcoma and carcinoma of the pancreas.

![Fig. 32 Market available anticancer drug.](image)

Moreover, various 2-aminoimidazole alkaloids including Naamine A [13], Naamine G [14], Girolline [15], Preclathridine A [16] have been isolated from marine sources [17-18], which were found to possess excellent anti-cancer activities (Figure 33). Further, some bis[2-chloroethyl]aminoimidazole derivatives have been found to act as DNA binding agents and topoisomerase II inhibitors [19].
2.1 Results & Discussion

A large number of 2-aminoimidazole derivatives have also been reported to possess diverse pharmacological properties such as antibacterial, anti-inflammatory and antiviral activity [20-22]. Similarly, quinoline and its derivatives have been described as another important class of pharmacologically active compounds that show broad spectrum of biological activities [23-24] such as antibacterial [25], antimalarial [26], anticancer [27], anti-inflammatory [28], antitumor [29], anti-HIV [30], antidepressant [31] and antiallergic [32]. Vesnarinone and linomide have been reported to be effective against MH-134 tumor cells and prostate cancer [33] (Figure 34).

2.1.1 Chemistry

Keeping in view of the above facts, it was thought of interest that a combination of imidazole and quinoline moiety may be more beneficial in inhibiting the cancer cells growth. The synthesis of 2-aminoimidazole-quinoline hybrids (182) is described below. To achieve the synthesis of target compounds (182), 2-chloroquinoline-3-carbaldehyde (178a) was prepared from by the Vilsmeier Haack reaction of acetonilide (177a) prepared by acetylation of aniline [34]. Similarly, 6-substituted-2-chloroquinoline-3-carbaldehyde (178b) was prepared starting from p-toluidine (Scheme-42).
The coupling of 2-chloroquinoline-3-carbaldehydes (178) and amino guanidine carbonate (179) was performed in a solution of hydrochloric acid and water under reflux that result the formation of corresponding hydrazones (180) (Scheme-43).

Scheme-43 Synthesis of 2-chloro-6-H/Mequinolinylhydrazinecarboximidamides.

The hydrazone (180) exists in cis and trans configuration. The cis configuration may be ruled out because of steric hindrance. However, the trans configuration of hydrazone exhibits rotational isomerism as shown in Figure 35. The rotational isomers (T-1 and T-2) are less stable in comparison to the trans configuration (T) probably due to presence of hydrogen bonding.

Fig. 35 Rotational isomers of compounds 180.
In $^1$H NMR spectrum of 180b, the characteristic most deshielded peak as a singlet at 8.96 ppm assigned to the proton 4-H, however, a singlet at 7.71 ppm was assigned to the proton 5-H. Two doublets at 7.78 ppm ($J = 8.2$ Hz) and 7.55 ppm ($J = 8.0$ Hz) were assigned to 7-H and 8-H of quinoline ring, respectively. Another two singlets due to -NH$_2$ at 5.86 ppm and -CH=N- proton at 8.29 ppm were observed and thus indicated the formation of hydrazone (180) (Figure 36, 37 and 38).

Treatment of either $\alpha$-bromoketones or $\alpha$-tosyloxyketones (181) with hydrazone (180) gave exclusively $E$-N-[(2-Chloro-6-substitutedquinolin-3-yl)methylene]-4-aryl]-1H-imidazole-1,2-diamines (182) in a regioselective manner (Scheme-44) instead of imidazole 183-185 (Figure 39).

\[ \text{Scheme-44 Synthesis of imidazole-quinoline hybrids (182a-p).} \]
Infact, the reaction of 180 with 181 may generate isomeric 2-aminoimidazoles 182 and 183 via path-A or path-B. Whereas through path-C, there is possibility of the formation of imidazoles 184 and 185.

Coming from all the discussed possibilities out, the reaction of 180 with 181 finally gave 182 exclusively. The plausible mechanistic pathways involved in the formation of 182 is summarized in scheme-45.

Scheme-45 A plausible mechanism involved in the formation of the compound (182).

The reaction of more stable trans-conformer of hydrazones (180) with either α-bromoketones or α-tosyloxyketones (181) resulted in the formation of 2-amino-4-arylimidazole derivatives (182) instead of other isomers was substantiated on the basis of analysis of advance 2D spectral data of the final product obtained.

Further, 2-aminoimidazole 182 may exhibit rotational isomerism due to rotation around N-N bond as shown in Figure 40. However, the structure of 182 as rotational isomer A instead of B was established on the basis of combined use of FT-IR, NMR (1H and 13C), 2D NMR spectroscopy and mass spectrometry.
Fig. 40 Possible rotational isomers of the compound 182.

In IR spectrum, the compound 182d exhibited two characteristic bands at 3415 and 3275 cm\(^{-1}\) due to asymmetric and symmetric N-H stretching vibration of \(-\text{NH}_2\) group, respectively attached to the position-2 of imidazole ring. The other bands have also been explained in experimental section.

Fig. 41 Chemical shifts of all protons of 182d.

In \(^1\text{H}\) NMR spectrum of 182d, the characteristic peak as a singlet at 8.30 ppm due to 5-H and another singlet at 6.50 ppm due to protons of \(-\text{NH}_2\) group attached at position-2 of imidazole ring supported the formation of imidazole nucleus. The presence of quinoline moiety in this hybrid compound was shown by the most deshielded proton 4'-H that appeared as a singlet at 9.38 ppm and two doublets at 8.09 ppm (\(J = 7.9\) Hz) and 8.01 ppm (\(J = 8.0\) Hz) were assigned to 5'-H and 8'-H. Further, a multiplet at 7.72-7.80 ppm and a triplet at 7.89 ppm were assigned to 6'-H and 7'-H, respectively of quinoline moiety. An important singlet at \(\delta 8.70\) ppm was assigned to the hydrogen of imine (\(-\text{CH}=\text{N}-\)) functionality and thus
showed the connectivity of imidazole and quinoline moieties. A doublet of two protons $3''$-H and $5''$-H at 7.42 ppm ($J = 8.4$ Hz) and a multiplet of $2''$-H and $6''$-H at 7.72-7.80 ppm were also observed (Figure 41 and 42).

In $^{13}$C NMR spectrum of 182d, carbon-2, 4 and 5 of the imidazole nucleus resonated at 150.6, 133.6 and 102.7 ppm, respectively.

Another important peak in $^{13}$C spectrum at 140.0 ppm was assigned to imine carbon (-N=CI), linkage between imidazole and quinoline nuclei (Figure 43 and 44). The above results were further supported by the information obtained from DEPT-135, HSQC and HMBC.

In DEPT-135 spectrum of 182d, methylene carbon was not observed whereas nine carbon signals were appeared due to nine methine carbons (Figure 45). The proton-carbon correlations were established by analyzing the HSQC spectrum of 182d and therefore, supported well the proposed structure (Figure 46 and 46').

HMBC gives the information of carbon atom having two and three-bond coupling with proton. In this 2D NMR spectrum, proton $5$-H attached with carbon C-5 showed two bond coupling with C-4 at 136.9 ppm and three-bond coupling with C-2 at 150.6. Similarly, proton $4'$-H attached with carbon C-4' showed two-bond coupling with C-3' at 126.3 ppm and three-bond coupling with C-2' at 149.0 ppm, respectively (Figure 47 and 47').

The connectivity of all the hydrogens in 182d either through bond or in space was performed with the help of COSY and ROESY spectrum (Figure 48).
Fig. 48 COSY, ROESY and HMBC correlations of 182d.

The COSY spectrum of 182d showed the following information about proton-proton correlations (Figure 48, 49 and 49').

- 3''-H & 5''-H (d, 7.42 ppm, J = 8.4 Hz) ↔ 2''-H & 6''-H (m, 7.72-7.80 ppm)
- 5'-H (d, 8.09 ppm, J = 7.9 Hz) ↔ 6'-H (m, 7.72-7.80 ppm)
- 6'-H (m, 7.72-7.80 ppm) ↔ 7'-H (m, 7.89 ppm)
- 7'-H (m, 7.89 ppm) ↔ 8'-H (d, 8.01 ppm, J = 8.0 Hz)

The ROESY spectrum clearly indicated the formation of one of the rotational isomers of 182, wherein 5-H and N=C-H hydrogens were found in close proximity, instead of isomeric compound 183-B. In ROESY spectrum, it was observed that hydrogen at position-5 of imidazole nucleus is closely associated in space with imine hydrogen (CH=N) and ortho hydrogens of p-chlorophenyl ring (Figure 48, 50 and 50').

There was no proximity between -NH₂ and imine hydrogen found in ROESY that established the orientation of amino group far from imine group. All the spectral data confirmed the formation of compound 182 with trans geometry having imine-imidazole hydrogen in close proximity instead of isomeric compound 182-B.
2.1.2 Biology

2.1.2a Cytotoxic activity against cancer and primary endothelial cells

All the synthesized compounds (182) were screened for their anti-cancer potential by evaluating cytotoxicity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using Doxorubicin (Figure 51) as the standard drug.

![Fig. 51 Doxorubicin.](image)

The toxicity profile of the compounds 182 on the proliferation of normal cell lines was also checked. The cytotoxicity studies were conducted against two human colon cancer cell lines (HCT-116, DLD-1), human breast cancer cell line (MDA-MB-231) and normal human cell line (HUVEC) at different concentrations i.e. 10, 20, 30, 40 and 50 µM up to 48 h (Figure 52-54).

The IC$_{50}$ value of all the synthesized compounds is presented in Table 1. The primary scaffold 182a (Imd-Ph) showed good cytotoxicity profile in colon cancer cell lines with IC$_{50}$ values around 6.92 µM and 16.37 µM against HCT-116 and DLD-1 cell lines, respectively (Table 1). Replacement of phenyl group at position-4 of imidazole ring in Imd-Ph (182a) with 1-naphthyl moiety (182k) preserved its anticancer activity in HCT-116 cell line (IC$_{50}$ = 6.34 µM) and improved its activity in DLD-1 cell line (IC$_{50}$= 9.48 µM). However, replacement of phenyl group with 2-naphthyl group (182l) diminished anticancer activity against HCT-116 and DLD-1 as indicated by their IC$_{50}$ values (>50 µM) in colon cancer cell lines. Similarly, attachment of 2-thienyl group (182o) in place of phenyl group in Imd-Ph (182a) causes no appreciable change in IC$_{50}$ value in HCT-116 (7.22 µM) while lowered the IC$_{50}$ value in DLD-1 (10.27 µM) cell line. Any change in the electronic environment of the phenyl ring of Imd-Ph (182a) with respect to electron donating groups (-Me, 182b; -OMe, 182c) and electron-withdrawing groups (-Cl, 182d; -Br, 182e) diminished the anticancer activity (IC$_{50}$ > 50 µM) in colon cancer cell lines. Introduction of methyl
group at position-6 of quinoline ring in Imd-Ph (182p) did not affect anticancer activity in HCT-116 cell line (7.12 µM) and lowered the IC$_{50}$ value in DLD-1 (8.79 µM) (Table 1).

Table 1. Cytotoxic profile of the synthesized compounds 182a-p.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HCT-116</th>
<th>DLD-1</th>
<th>MDA-MB-231</th>
<th>HUVEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imd-Ph</td>
<td>182a</td>
<td>6.92 ± 2.22</td>
<td>16.37 ± 3.37</td>
<td>49.04 ± 2.87</td>
</tr>
<tr>
<td>Imd-MePh</td>
<td>182b</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-OMePh</td>
<td>182c</td>
<td>31.82 ± 5.82</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-ClPh</td>
<td>182d</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-BrPh</td>
<td>182e</td>
<td>&gt;50</td>
<td>23.94 ± 5.55</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-Me-Ph</td>
<td>182f</td>
<td>7.12 ± 4.74</td>
<td>8.79 ± 1.86</td>
<td>48.14 ± 1.64</td>
</tr>
<tr>
<td>Imd-Me-MePh</td>
<td>182g</td>
<td>39.59 ± 0.42</td>
<td>25.22 ± 0.77</td>
<td>22.27 ± 5.41</td>
</tr>
<tr>
<td>Imd-Me-OMePh</td>
<td>182h</td>
<td>&gt;50</td>
<td>25.71 ± 7.84</td>
<td>23.55 ± 2.81</td>
</tr>
<tr>
<td>Imd-Me-C1Ph</td>
<td>182i</td>
<td>&gt;50</td>
<td>38.90 ± 1.08</td>
<td>35.16 ± 2.47</td>
</tr>
<tr>
<td>Imd-Me-BrPh</td>
<td>182j</td>
<td>49.92 ± 1.34</td>
<td>39.29 ± 2.67</td>
<td>38.21 ± 8.98</td>
</tr>
<tr>
<td>Imd-1Nph</td>
<td>182k</td>
<td>6.34 ± 3.77</td>
<td>9.48 ± 3.45</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-2Nph</td>
<td>182l</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-Me-1Nph</td>
<td>182m</td>
<td>5.84 ± 1.11</td>
<td>8.30 ± 3.61</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-Me-2Nph</td>
<td>182n</td>
<td>27.39 ± 2.30</td>
<td>10.17 ± 5.17</td>
<td>35.55 ± 0.61</td>
</tr>
<tr>
<td>Imd-The</td>
<td>182o</td>
<td>7.22 ± 4.20</td>
<td>10.27 ± 8.97</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Imd-Me-The</td>
<td>182p</td>
<td>7.71 ± 3.20</td>
<td>8.99 ± 0.50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Dox</td>
<td>0.34 ± 0.03</td>
<td>0.84 ± 0.05</td>
<td>1.47 ± 0.22</td>
</tr>
</tbody>
</table>

Similarly, the result of anticancer activities of compounds 182m and 182p were found unaltered with methyl group introduction on quinoline ring as compared to compounds 182k and 182o, respectively. Replacement of hydrogen by methyl group in quinoline ring in compounds having phenyl group with electron-withdrawing -Cl (182i), -Br (182j) and electron-donating -Me (182g), and -OMe (182h) substituents causes increase in
IC$_{50}$ values thereby lowered their anticancer potential. The graphical representation of % cell survival (%) vs concentration (µM) against HCT-116 is provided in Figure 52a, b and c and against DLD-1 is provided in Figure 52d, e and f.

![Graphs](image-url)

**Fig. 52**

It has also been observed that neither the primary scaffold Imd-Ph (182a) nor its modified derivatives such as 182k, 182l, 182o were found active (IC$_{50}$ > 50 µM) against breast cancer cell line (MDA-MB-231). Introduction of methyl group in quinoline ring
(182f, 182m, 182n, 182p) as well as any change in the electronic environment of the phenyl group attached at position-4 of imidazole ring causes increase in IC$_{50}$ values and thus lowered their anticancer activities (Table 1). The graphical presentation of cell survival (%) vs concentration (µM) against MDA-MB-231 is given in Figure 53.

![Graphs showing cell survival vs concentration](image)

**Fig. 53**

These results indicated that aminimidazole-quinoline hybrid scaffold shows colon selective anticancer activities in comparison to the standard drug, doxorubicin, which showed no selectivity between different cancer types.

Interestingly, Imd-Ph (182a), Imd-Me-Ph (182f) and its naphthyl (182k, 182l) modifications were found non-toxic to primary (normal) cell line (HUVEC) (Figure 54, Table 1). Compounds with methyl group on quinoline ring (182g, 182h, 182i, 182j, 182m, 182n) as well as para substitution on phenyl group attached at position-4 of imidazole nucleus resulted in decrease in IC$_{50}$ values (10-30 µM) thereby increased the toxicity to normal cell lines. Collectively, these results indicated that the primary scaffold moiety is non-toxic to normal cell lines whereas its structural cognomers are toxic to normal cell line (HUVEC) (Table 1). The graphical presentation of cell survival (%) vs concentration (µM) against HUVEC is shown in Figure 54.
2.1.2b Structure activity-relationship (SAR)

Effect of substituents on position-4 of imidazole ring: On the basis of the results, it has been concluded that Imd-Ph (182a) shows selective toxicity against colon cancer cells and has relatively non-toxic to breast cancer cells as well as normal cells (Figure 56a). Moreover, presence of 1-naphthyl substituent instead of 2-naphthyl moiety is responsible for increased selective anticancer activity. Replacement of phenyl group by classical ring equivalent bioisostere such as thienyl moiety at position-4 of imidazole ring also affect the
anticancer activity and is found to be more effective as compared to substituted aryl moiety but less selective to cancer cells. On the other hand, any substitution on phenyl ring at position-4 leads to decrease in anticancer potential and increase in toxicity to normal cells (Figure 56c and 56d).

**Effect of methyl group introduction at position-6 of quinoline ring:** Replacement of hydrogen atom by the methyl group at position-6 of quinoline ring leads to significant increase in cytotoxicity (Figure 56b and 55).

![Figure 56](image-url)
Fig. 37 $^1$H NMR spectrum of compound 180b.
Fig. 38 $^{13}$C NMR spectrum of compound 180b.
Fig. 42 $^1$H NMR spectrum of compound 182d.
Fig. 44 $^{13}$C NMR spectrum of compound 182d.
Fig. 45 DEPT-135 NMR spectrum of compound 182d.
Fig. 46 HSQC NMR spectrum of compound 182d.
Fig. 46' Expanded HSQC NMR spectrum of compound 182d.
Fig. 47 HMBC NMR spectrum of compound 182d.
Fig. 47' Expanded HMBC NMR spectrum of compound 182d.
Fig. 49 COSY NMR spectrum of compound 182d.
Fig. 49' Expanded COSY NMR spectrum of compound 182d.
Fig. 50 ROESY NMR spectrum of compound 182d.
Fig. 50' Expanded ROESY NMR spectrum of compound 182d.
REFERENCES

Section-2.1

Results & Discussion


SECTION-2.2

Synthesis of Some Novel 2-Aminobenzimidazole-Schiff base Hybrids as Anticancer Agents
Synthesis of Some Novel 2-Aminobenzimidazole-Schiff base hybrids as Anticancer Agents

Benzimidazole ring constitutes an important class of biologically active heterocycles that attracted a great attention of medicinal chemists due to their broad spectrum of pharmacological properties. In order to combat various diseases with minimal toxicity and maximal effects many researchers have synthesized a large number of drugs containing benzimidazole nucleus. The benzimidazole moiety is associated with a wide range of biological properties such as anticancer [1, 2], antiviral [3, 4], antibacterial [5-7], antifungal [8, 9], anthelmintic [10], antioxidant [11, 12], antihypertensive [13] and anticoagulant [14] activity.

2-Aminobenzimidazole is widely found as a core pharmacophoric unit in commercial drugs such as Vermox, Fenbendazole, Oncodazole, Mizolastine, Enviroxime, and Benomyl (Figure 57) and thus plays an important role in pharma sectors. Compounds bearing this unit have been found to possess antilipidemic or platelet antiaggregatory [15], antimicrobial [16], antiinflammatory [17], antiHIV [18], immunosuppressive and antiviral activity [19]. Most of the compounds display affinity at the benzodiazepine receptor [20]. Some of them are selective inhibitors of nitric oxide [21] and the neuronal calcium channel blockers [22].

On the other hand, Schiff’s bases functionality (-N=CH) also acts as an important pharmacophore and is known to exhibit antibacterial [23], antimycobacterial [24], antitumor
[25], antileishmanial activity [26]. The combination of two pharmacophores on the same scaffold leading to hybrid molecules or conjugates as the more bioactive agents has become a center of great interest in the field of medicinal chemistry.

2.2.1 Chemistry

To achieve the synthesis of target compounds (196), 1,2-diaminobenzimidazole, 4-formylpyrazole and indole-3-carbaldehyde were prepared. There are numerous methods available in literature to obtain 1,2-diaminobenzimidazole [27, 28]. However, the method used to prepare 1,2-diaminobenzimidazole (187) involves N-amination of 2-aminobenzimidazole (186) with hydroxylamine-\(O\)-sulfonic acid as shown in Scheme-46 [29].

![Scheme-46 Synthesis of 1,2-Diaminobenzimidazole.](image)

In order to synthesize various 4-formylpyrazoles (191), firstly hydrazones (190) were obtained by the condensation of 4-substituted acetophenones (188) and phenylhydrazine (189) in ethanol in presence of a catalytic amount of acetic acid. The hydrazones (190) were then cyclized via Vilsmeir-Haack reaction [30] (Scheme-47).

![Scheme-47 Synthesis of N-Phenyl-3-aryl-4-formylpyrazoles.](image)

2-Substituted indoles (192) were methylated with methyl iodide in presence of iodomethane in presence of sodium hydride and dimethylformamide to produce N-methyl-2-substituted-3-formyl indoles (193) which were further treated with DMF-POCl3 to afford indol-3-aldehydes (194) [31, 32] (Scheme-48).
Scheme-48 Synthesis of N-Methyl-2-substituted-3-formyl indoles.

The treatment of 1,2-diaminobenzimidazole (187) with various aldehydes (191, 194 and 195) gave (E)-N-substituted-1H-benzo[d]imidazole-1,2-diamines (196) exclusively as outlined in Scheme-49.

Scheme-49 Synthesis of imidazole-Schiff base hybrids (196a-s).

The coupling of 187 and 191a may generate four isomers of 196a as a result of geometrical and conformational isomerism as shown in Figure 58. These four isomeric Schiff bases (A, B, C and D) may be formed exclusively due to their more stable trans
geometry (E) where two bulky groups lie anti to each other. The formation of other conformers having *cis* orientation was ruled out on the basis of their less stable structure where two bulky groups may induce steric repulsion. Actually, among four possible isomers of 196a, the formation of D was substantiated on the basis of analysis of advance 2D NMR spectral data.

![Four possible conformers of 196a.](image)

The structure of 196 and assignments of protons and carbons for the conformer D have been established on the basis of FT-IR, $^1$H, $^{13}$C NMR and 2D NMR (COSY, ROESY and HSQC) spectroscopic and mass spectrometry techniques.

In IR spectrum, compound 196a exhibited two characteristic bands at 3448 and 3317 cm$^{-1}$ due to N-H asymmetric and symmetric stretching vibrations of $\text{-NH}_2$ group attached to the position-2 of benzimidazole ring and another characteristic band appeared at 1656 cm$^{-1}$ was assigned to imine (\text{-N=CH-}) connectivity between benzimidazole and pyrazole nucleus.
Fig. 59 Assignment of protons of the compound 196a

In $^1$H NMR spectrum of 196a, a characteristic peak was appeared as a singlet at $\delta$ 6.73 ppm due to -NH$_2$ group present on position-2 of benzimidazole nucleus, whereas a singlet of one proton of pyrazole moiety attached at position-5 was appeared at $\delta$ 9.37 ppm. Appearance of another important singlet at $\delta$ 8.95 ppm due to -N=CH- proton further supported the formation of benzimidazole-pyrazole hybrid molecule (Figure 59 and 60). The COSY spectrum of the compound 196a indicated the following H-H correlations (Figure 62 and 62$^\prime$):

- 7-H (d, $\delta$ 7.53 ppm, $J$ = 8.4 Hz) ↔ 6-H (m, $\delta$ 6.97-7.00 ppm)
- 6-H (m, $\delta$ 6.97-7.00 ppm) ↔ 5-H (m, $\delta$ 7.05-7.09 ppm)
- 5-H (m, $\delta$ 7.05-7.09 ppm) ↔ 4-H (d, $\delta$ 7.23 ppm, $J$ = 7.2 Hz)
- 2$''$/6$''$-H (d, $\delta$ 7.83 ppm, $J$ = 7.6 Hz) ↔ 3$''$/5$''$-H (d, $\delta$ 7.78 ppm, $J$ = 8.0 Hz)
- 2$''$/6$''$-H (d, $\delta$ 7.97 ppm, $J$ = 7.6 Hz) ↔ 3$''$/5$''$-H (m, $\delta$ 7.59-7.63 ppm)
- 3$''$/5$''$-H (m, $\delta$ 7.59-7.63 ppm) ↔ 4$''$-H (m, $\delta$ 7.42-7.45 ppm)

The ROESY spectrum clearly indicated the formation of conformer D wherein proton 7-H is correlated with the proton N=C-H. Furthermore, the protons, N=C-H and 2$''$/6$''$-H were found to be correlated in space. This type of arrangement and correlation of protons are not possible in other three possible conformers (A, B and C). In the ROESY spectrum, it has also been observed that proton present at position-5$'$ of pyrazole nucleus is closely associated in space with 2$''$-H and 6$''$-H of phenyl ring. Moreover, -N=CH-functional group was also found near to 7-H proton of benzimidazole and 2$''$-H and 6$''$-H of
bromine substituted phenyl ring. There was no closeness found between -NH$_2$ and imine protons, which established the orientation of amino group far from imine group. The 5'-H of pyrazole ring was not showing spatial interaction with imine proton shown in Figure 63 and 63'. In conclusion, all these results directed us to consider the D conformer of 196a as a final product.

![Diagram of COSY and ROESY correlations of 196a.](image)

The formation of compound 196a was further supported on the basis of their $^{13}$C NMR spectral data in which carbon of -N=CH- group resonated at 141.26 ppm and carbon-5' of pyrazole and carbon-7 of benzimidazole resonated at 129.61 and 110.15 ppm, respectively (Figure 64).

The correlations between carbon-hydrogen of 196a drawn from HSQC spectral data further supported the structure of final compounds. Some important correlations were (Figure 65 and 65'):

- 8.95 ppm (N=C-H) ↔ 141.26 ppm (N=C-H)
- 9.37 ppm (5'-H) ↔ 129.61 ppm (C-5')
- 7.53 ppm (7-H) ↔ 110.15 ppm (C-7)
2.2.2 Biology

2.2.2a Anticancer activity (% cell viability)

To study the *in vitro* cytotoxic effects of the compounds 196a-s, standard MTT assay against MCF-7, MRC-5 and MCF-10A cells lines was applied for 24 h exposure. The percentage of DMSO (0.1%) used in the experiment did not affect the growth of the cells and also used as control. It has been observed that among all the synthesized compounds, 196n, 196o and 196p exhibited higher cytotoxic potential against all the three cell lines. Compound 196o showed 22.36 ± 0.41, 23.34 ± 4.24 and 22.19 ± 6.39 % cell viability of MCF-7, MRC-5 and MCF-10A cell lines, respectively. Compound 196p (28.63 ± 2.04, 25.71 ± 6.27 and 23.22 ± 5.99 % cell viability of MCF-7, MRC-5 and MCF-10A cell lines, respectively) and 196n (32.80 ± 3.81, 28.59 ± 2.81 and 26.72 ± 4.46 % cell viability of MCF-7, MRC-5 and MCF-10A cell lines, respectively) were showing cytotoxic potential near to the compound 196o. Among the synthesized compounds, least toxicity was recorded for the compound 187 having 81.5 ± 2.40, 75.51 ± 3.80 and 80.28 ± 7.50 % cell viability of MCF-7, MRC-5 and MCF-10A cell lines, respectively. The resulting protocol has shown that *in vitro* anticancer activity of (E)-N-substituted-1H-benzo[d]imidazole-1,2-diamine derivatives exhibited significant cytotoxicity against three carcinoma cells (MCF-7, MRC-5 and MCF-10A). It is clear from the data that the compound 196o has the highest level of cytotoxicity potential (Figure 66a-c).

![Cell viability assay on MCF-7](image-url)
Fig. 66a-c In vitro cells viability (% cytotoxicity) of the compounds (196a-s) at 40 µM against a) MCF-7 cell lines, b) MRC-5 cell lines and c) MCF-10A cell lines by MTT assay at 24 h of exposure.
Table 2. Anti-cancer activity (% cell viability) of \((E)-N\)-substituted-1H- benzo[d]imidazole-1,2-diamines (187 and 196a-s).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cell lines</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7</td>
<td>MRC-5</td>
<td>MCF-10A</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±2</td>
<td>100±2</td>
<td>100±2</td>
<td></td>
</tr>
<tr>
<td>187</td>
<td>81.5 ± 2.40*</td>
<td>75.51 ± 3.80</td>
<td>80.28 ± 7.50</td>
<td></td>
</tr>
<tr>
<td>196a</td>
<td>44.76 ± 9.20*</td>
<td>40.96 ± 6.40*</td>
<td>47.93 ± 7.30*</td>
<td></td>
</tr>
<tr>
<td>196b</td>
<td>34.46 ± 4.19*</td>
<td>48.19 ± 6.05*</td>
<td>37.55 ± 8.66*</td>
<td></td>
</tr>
<tr>
<td>196c</td>
<td>63.53 ± 3.02*</td>
<td>63.98 ± 9.22*</td>
<td>62.24 ± 3.00*</td>
<td></td>
</tr>
<tr>
<td>196d</td>
<td>48.70 ± 3.20*</td>
<td>47.05 ± 6.32*</td>
<td>69.56 ± 5.05*</td>
<td></td>
</tr>
<tr>
<td>196e</td>
<td>56.63 ± 10.13*</td>
<td>56.57 ± 8.64*</td>
<td>61.88 ± 9.61*</td>
<td></td>
</tr>
<tr>
<td>196f</td>
<td>52.56 ± 2.43*</td>
<td>51.21 ± 7.78</td>
<td>45.87 ± 5.65*</td>
<td></td>
</tr>
<tr>
<td>196g</td>
<td>52.56 ± 3.57*</td>
<td>52.46 ± 3.57*</td>
<td>46.23 ± 6.61*</td>
<td></td>
</tr>
<tr>
<td>196h</td>
<td>57.00 ± 6.48*</td>
<td>52.74 ± 5.03*</td>
<td>47.17 ± 5.56*</td>
<td></td>
</tr>
<tr>
<td>196i</td>
<td>51.03 ± 7.57*</td>
<td>39.25 ± 7.53*</td>
<td>49.09 ± 9.19*</td>
<td></td>
</tr>
<tr>
<td>196j</td>
<td>63.53 ± 3.02*</td>
<td>50.93 ± 10.98*</td>
<td>59.50 ± 8.91*</td>
<td></td>
</tr>
<tr>
<td>196k</td>
<td>67.76 ± 9.08*</td>
<td>66.94 ± 5.60*</td>
<td>72.41 ± 7.94*</td>
<td></td>
</tr>
<tr>
<td>196l</td>
<td>51.40 ± 2.90*</td>
<td>47.44 ± 4.06*</td>
<td>51.92 ± 9.77*</td>
<td></td>
</tr>
<tr>
<td>196m</td>
<td>59.20 ± 7.44*</td>
<td>51.48 ± 5.49*</td>
<td>55.91 ± 4.55*</td>
<td></td>
</tr>
<tr>
<td>196n</td>
<td>32.80 ± 3.81**</td>
<td>28.59 ± 2.81**</td>
<td>26.72 ± 4.46**</td>
<td></td>
</tr>
<tr>
<td>196o</td>
<td>22.36 ± 0.41**</td>
<td>23.34 ± 4.24**</td>
<td>22.19 ± 6.39**</td>
<td></td>
</tr>
<tr>
<td>196p</td>
<td>28.63 ± 2.04**</td>
<td>25.71 ± 6.27**</td>
<td>23.22 ± 5.99**</td>
<td></td>
</tr>
<tr>
<td>196q</td>
<td>65.26 ± 2.90*</td>
<td>40.71 ± 7.91*</td>
<td>52.93 ± 7.97*</td>
<td></td>
</tr>
<tr>
<td>196r</td>
<td>64.66 ± 5.76*</td>
<td>69.39 ± 4.41*</td>
<td>57.36 ± 9.78*</td>
<td></td>
</tr>
<tr>
<td>196s</td>
<td>57.83 ± 2.92*</td>
<td>60.98 ± 7.20*</td>
<td>46.73 ± 8.45*</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed in terms of mean ± SEM; n = 5 values are statistically significant at *p < 0.05 and higher significant at **p < 0.01; † denoted as not significant.
2.2.2b Structure activity-relationship (SAR)

![Diagram of structure activity-relationship (SAR)]

From the present investigation, some generalizations came into account as shown in Fig. 67.

i. Compounds 196 having substituted phenyl ring (when R = aryl) were found to be the more active than substituted indole and pyrazole derivatives against MCF-7, MRC-5 and MCF-10A cell lines.

ii. Substitution on position-3 or 4 of the phenyl ring (when R = aryl) shows interesting result with methoxy group but deprotection and protection of oxygen other than methyl group leads to decrease its anticancer potential.

iii. Among the synthesized compounds, 1960 was emerged as an excellent anticancer agent against all the three cell lines.

iv. Although mechanism is not clear, the results revealed that insertion of methoxy group at meta and para position of phenyl ring linked to –N=CH- of imidazole is responsible for good anticancer potential.

v. Electron releasing groups increase the activity while electron withdrawing groups decrease the activity in all cases.
Fig. 60 $^1$H NMR spectrum of compound 196a.
Fig. 62 COSY NMR spectrum of compound 196a.
Fig. 63 ROESY NMR spectrum of compound 196a.
Fig. 63' Expanded ROESY NMR spectrum of compound 196a.
Fig. 64 $^{13}$C NMR spectrum of compound 196a.
Fig. 65 HSQC NMR spectrum of compound 196a.
Fig. 65' Expanded HSQC NMR spectrum of compound 196a.
Section-2.2  Results & Discussion

REFERENCE


Section-2.2 Results & Discussion


SECTION-2.3

Synthesis of Some Novel N-ARYL-2-Mercaptoimidazoles as Potential Antimicrobial and Antioxidant Agents
Synthesis of Some Novel N-Aryl-2-Mercaptoimidazoles as Potential Antimicrobial and Antioxidant Agents

Heterocyclic compounds based on azole moiety, in general, are found of great importance due to their wide range of applications in synthetic and medicinal chemistry [1-4]. They exhibit various biological activities such as antibacterial [5-7], antiinflammatory [8-10], anticancer [11, 12], antiviral [13], antiobesity [14] and antiamoebic activity [15, 16]. Among azoles, imidazole has proven itself of a high therapeutic indexed nucleus in the recent past. Imidazole moiety has been frequently adopted by a large number of biologically active compounds as anticancer [17], antimicrobial [18, 19] antibacterial [20], antifungal [21, 22], \(\beta\)-lactamase inhibitors [23] and antioxidant agents [24].

Although, various imidazoles based antimicrobial drugs are available in the market (Figure 68), a serious matter of concern in the treatment of microbial infections is an emergence of resistance against these drugs [25]. Many of currently available drugs are toxic and enable recurrence because of their bacteriostatic/fungistatic nature instead of bactericidal/fungicidal or lead to emergence of resistance after a prolonged period of...
administration [26]. Therefore, increasing resistance of microorganisms to currently available antimicrobial drugs is the major cause of morbidity and mortality throughout the world. Several pathogenic agents established their virulence and pathogenicity by virtue of their ability to produce free radicals and damage the cells of the immune system. Recent reports have demonstrated that compounds like minocycline, arctigenin, fenofibrate, and curcumin show protection against infection and were also found potent free radical scavengers and antioxidants [27, 28].

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. They may play an important role in the treatment or prevention of various degenerative or chronic diseases such as atherosclerosis, brain dysfunction, immune system decline and cancer, etc. [29-32]. Thus development of novel potential leads having antimicrobial and antioxidant properties is still in demand. Viewing above facts and in continuation of our work related to discover new biologically active azoles, some novel N-substituted mercaptoimidazole derivatives have been synthesized as potential antioxidant and antimicrobial agents.

2.3.1 Chemistry
The synthesis of some novel 2-mercaptoimidazoles has been carried out as shown in Scheme 50.

![Scheme 50](image_url)

Scheme-50 Synthesis of 2-mercaptoimidazoles.

To achieve target compounds, firstly, the reaction of 3-aminoacetophenone (197) was performed with various phenacyl bromides (181) to obtain the corresponding anilino...
compounds (198), key intermediates for the final compounds. Further, the treatment of 198 with potassium thiocyanate gave 1-[3-(2-mercapto-aryl-1H-imidazol-1-yl)phenyl]ethanones (199) exclusively. The plausible mechanistic steps involved in formation of 199 are summarized in Scheme 51.

![Scheme 51](image)

Scheme-51 A plausible mechanism involved in the formation of the compound 199.

In principle, cyclization of 198 may give two conformationally different 2-mercaptoimidazoles (199A and 199B). The formation of 199A was confirmed on the basis of advance 2D NMR spectral data of the final product (Figure 69).

![Fig. 69](image)

Fig. 69 Possible rotational isomers of the compound 199.

The structures of the products (199) were established on the basis of a combined use of FT-IR, NMR (1H, 13C & 2-D) NMR and mass spectral data.

In IR spectrum, compound 199 showed a characteristic band at 2735 cm\(^{-1}\) due to S-H stretching vibration of mercapto group attached at position-2 of the imidazole ring. Another characteristic band at 1685 cm\(^{-1}\) was appeared due to carbonyl stretch. The other band are also been explained via experimental.
In $^1$H NMR spectrum, an appearance of a characteristic signal as a singlet at 8.29 ppm due to 5-H confirmed the formation of imidazole nucleus. Another singlet at $\delta$ 12.99 ppm was assigned to the proton of S-H group which supported the formation of 2-mercaptoimidazole moiety [33]. Two doublets at 7.25 ppm ($J = 7.8$ Hz) and 7.67 ppm ($J = 7.9$ Hz) were assigned to the protons (3''-H & 5''-H) and (2''-H & 6''-H), respectively. Besides this, two multiplets at 7.68-7.71 ppm and 8.00-8.04 ppm were assigned to 5'-H and (4'-H/6'-H), respectively (Figure 70 and 71).

![Fig. 70 Assignments of all protons of the compound 199b.](image)

In $^{13}$C NMR spectrum of 199b carbon-2, 4 and 5 of the imidazole nucleus resonated at 162.9, 125.7 and 125.3 ppm, respectively (Figure 72 and 73). The above results were further supported by the information obtained from HMBC and HSQC.

The key correlations between carbon-hydrogen drawn from HSQC spectra were (Figure 74 and 74'):
### Results & Discussion

- **8.29 ppm (5'-H)** ↔ 125.33 ppm (C-5)
- **7.90 ppm (2'-H)** ↔ 115.63 ppm (C-2')
- **7.67 ppm (2''-H and 6''-H)** ↔ 124.74 ppm (C-2'' and C-6'')
- **7.25 ppm (3''-H and 5''-H)** ↔ 129.95 ppm (C-3'' and C-5'')
- **7.68-7.71 ppm (5'-H)** ↔ 129.71 ppm (C-5')

In HMBC spectrum, proton 5'-H attached with carbon C-5 showed two bond coupling with C-4 at 125.7 ppm. Similarly, proton 2'-H attached with carbon C-2' showed two-bond coupling with C-1' at 138.4 ppm and three-bond coupling with C-6' at 130.7 ppm, respectively (Figure 75 and 75').

The connectivity of all the hydrogens in 199b either through bond or in space was performed with the help of COSY and ROESY spectrum (Figure 76).

![COSY and ROESY correlations of 199b](image)

**Fig. 76** COSY and ROESY correlations of 199b.

The COSY spectrum of 199b showed the following information about proton-proton correlations (Figure 76, 77 and 77'):
- **3''-H & 5''-H** (d, 7.25 ppm, \( J = 7.8 \) Hz) ↔ **2''-H and 6''-H** (d, 7.67, \( J = 7.9 \) Hz)
- **4'-H & 6'-H** (8.02-8.04 ppm) ↔ **5'-H** (7.69-7.71 ppm)

The ROESY spectrum of the final product clearly indicates the formations of 199A where S-H of imidazole and 2'-H proton of phenyl ring were found to lie close to each other in the space. This arrangement of protons is not possible in other product (199B). In 199B, there should be an appearance of signal corresponding to spatial closeness relationship between S-H and 6'-H protons in ROESY spectrum, which actually was not observed (Figure 76 and 78). In conclusion, spectral results directed us to consider the structure 199A for the final product 199.

The hydroxyl compounds 200 were obtained from reduction of 199 by NaBH₄ in presence of aqueous ethanol. The structure of the compound 200 was established on the...
basis of a combined use of FT-IR, NMR ($^1$H & $^{13}$C) and mass spectral data. In IR spectrum of compound 200b, two characteristic bands at 3496 cm$^{-1}$ and 2731 cm$^{-1}$ were assigned to -OH and SH group. In IR spectrum of 200b, characteristic band at (1685 cm$^{-1}$) of carbonyl group was found to be disappeared and a new band at (3496 cm$^{-1}$) due to -OH stretching was observed which indicate the formation of hydroxyl compounds 200b. In $^1$H NMR spectrum, an appearance of a characteristic signal appeared as a doublet at 5.30 ppm ($J = 4$ Hz) assigned to -OH group also confirmed the reduction of carbonyl group. Another important signal which supports the formation of hydroxyl derivative (200b) was an appearance of a doublet due to methyl proton at 1.38 ppm ($J = 6.4$ Hz). A proton 1'''-H adjacent to methyl proton was observed as multiplet at 4.78-4.81 ppm (Figure 79 and 80).

\[ \text{Fig. 79 Assignments of protons of the compound 200b.} \]

The formation of 200 was further supported on the basis of $^{13}$C NMR spectral data. In $^{13}$C NMR spectrum of 200b, carbon-2, 4 and 5 of the imidazole nucleus resonated at 162.8, 115.8, and 125.4 ppm, respectively. Carbonyl carbon C-1''' resonated initially at 197.7 in spectrum of 199b was found to be disappeared and an appearance of a new upfield carbon signal at 68.2 ppm due to C-1''' was observed on the conversion of 199 into 200 (Figure 80 and 81).
These results were further supported by the 2D-NMR HSQC and HMBC (Figure 82, 82', 83 and 83') spectral data. Some important correlations observed from HSQC spectral data were:

- 7.77 ppm (5-H) $\leftrightarrow$ 125.4 ppm (C-5)
- 7.63 ppm (2'-H) $\leftrightarrow$ 123.1 ppm (C-2')
- 7.66 ppm (2''-H/6''-H) $\leftrightarrow$ 124.7 ppm (C-2''/C-6'')
- 7.23 ppm (3''-H/5''-H) $\leftrightarrow$ 129.9 ppm (C-3''/C-5'')
- 7.46 ppm (5'-H) $\leftrightarrow$ 128.8 ppm (C-5')
- 1.38 ppm (1'''-CH$_3$) $\leftrightarrow$ 26.3 ppm (1'''-CH$_3$)
- 5.30 ppm (1'''-H) $\leftrightarrow$ 68.2 ppm (1'''-H)

The COSY spectrum of 200b showed the information about proton-proton correlations (Figure 84 and 84'):

- 3''-H/5''-H (d, 7.23 ppm, $J = 7.9$ Hz) $\leftrightarrow$ 2''-H/6''-H (d, 7.67 ppm, $J = 7.9$ Hz)
- 4'-H (d, 7.40 ppm, $J = 7.56$ Hz) $\leftrightarrow$ 5'-H (7.44-7.48 ppm)
- 5'-H (7.44-7.48 ppm) $\leftrightarrow$ 6'-H (d, 7.57 ppm, $J = 7.7$ Hz)
- 1'''-CH$_3$ (1.38 ppm) $\leftrightarrow$ 1'''-H (5.30 ppm)
The ROESY spectrum of the hydroxyl compound (200b) clearly indicated the formation of 200A, where O-H proton attached at phenyl ring was found to lie close to S-H and 2'-H proton in the space (Figure 85 and 86). This arrangement of protons is not possible in other product (200B). In 200B, there should be an appearance of signal corresponding to spatial closeness relationship between S-H and O-H protons in ROESY spectrum, which actually was not observed. In conclusion, spectral results directed us to consider the structure 200A for the final product 200.
2.3.2 BIOLOGY

2.3.2a Antioxidant activity

Antioxidant profile of all synthesized compounds were tested at different concentrations and checked their EC$_{50}$ value by DPPH method. Ascorbic acid was used as a reference control. Tested compounds possessed good antioxidant profile, nearly same and in some cases more than ascorbic acid.

Table 3. (The *in vitro* free radical-scavenging activity of the synthesized compounds, measured by DPPH assay).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentrations (µg/mL)</th>
<th>EC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>199a</td>
<td>86.69 ± 0.19</td>
<td>86.23 ± 0.10</td>
</tr>
<tr>
<td>199b</td>
<td>86.67 ± 0.05</td>
<td>86.25 ± 0.17</td>
</tr>
<tr>
<td>199c</td>
<td>86.49 ± 0.07</td>
<td>86.31 ± 0.05</td>
</tr>
<tr>
<td>199d</td>
<td>86.41 ± 0.02</td>
<td>86.24 ± 0.05</td>
</tr>
<tr>
<td>199e</td>
<td>87.14 ± 0.03</td>
<td>86.41 ± 0.05</td>
</tr>
<tr>
<td>199f</td>
<td>86.50± 0.05</td>
<td>86.33 ± 0.02</td>
</tr>
<tr>
<td>199g</td>
<td>86.30± 0.09</td>
<td>86.35 ± 0.07</td>
</tr>
<tr>
<td>200a</td>
<td>86.30 ± 0.84</td>
<td>81.39 ± 0.05</td>
</tr>
<tr>
<td>200b</td>
<td>86.29 ± 0.02</td>
<td>86.03 ± 0.03</td>
</tr>
<tr>
<td>200c</td>
<td>86.44 ± 0.10</td>
<td>86.18 ± 0.03</td>
</tr>
<tr>
<td>200d</td>
<td>86.39 ± 0.20</td>
<td>86.12 ± 0.02</td>
</tr>
<tr>
<td>200e</td>
<td>86.42 ± 0.03</td>
<td>86.31 ± 0.03</td>
</tr>
<tr>
<td>200f</td>
<td>86.34 ± 0.13</td>
<td>86.28 ± 0.05</td>
</tr>
<tr>
<td>200g</td>
<td>85.42 ± 0.07</td>
<td>86.81 ± 0.06</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>87.83 ± 0.03</td>
<td>87.51 ± 0.08</td>
</tr>
</tbody>
</table>

Values expressed as % age DPPH radical scavenging activity, as means of three replicates ± SD

On the basis of EC$_{50}$ values shown in Table 3, the compound 199a was found four-fold more active than the standard compound 3.125-6.25 µg/mL. The result revealed that...
there was not any significant change in activity observed on conversion of carbonyl into hydroxyl group. In carbonyl derivatives 199a-f, unsubstituted derivatives were found active than substituted compounds. On the other hand, all hydroxyl derivatives were not found to show any significant difference in antioxidant activity and lie in the range of 6.25-12.5 µg/mL.

### 2.3.2b Antimicrobial activity

In the present study, all synthesized compounds were screened for their antibacterial and antifungal activity. Tested compounds possessed variable antibacterial activity against the Gram-positive (*S. aureus* and *B. subtilis*) bacteria whereas none of the compounds displayed inhibitory potential against Gram-negative bacteria.

**Table 4. In vitro antimicrobial activity of chemical compounds through agar well diffusion method.**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Diameters of growth of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>199a</td>
<td>13.6</td>
</tr>
<tr>
<td>199b</td>
<td>12.3</td>
</tr>
<tr>
<td>199c</td>
<td>12.6</td>
</tr>
<tr>
<td>199d</td>
<td>12.3</td>
</tr>
<tr>
<td>199e</td>
<td>13.3</td>
</tr>
<tr>
<td>199f</td>
<td>13.3</td>
</tr>
<tr>
<td>199g</td>
<td>12.6</td>
</tr>
<tr>
<td>200a</td>
<td>17.3</td>
</tr>
<tr>
<td>200b</td>
<td>13.6</td>
</tr>
<tr>
<td>200c</td>
<td>15.3</td>
</tr>
<tr>
<td>200d</td>
<td>14.6</td>
</tr>
<tr>
<td>200e</td>
<td>12.6</td>
</tr>
<tr>
<td>200f</td>
<td>13.5</td>
</tr>
<tr>
<td>200g</td>
<td>12.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26.6</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>Nt</td>
</tr>
</tbody>
</table>

- No activity; * Values, including diameter of the well (8 mm), are means of three replicates

In case of yeast, some of the compounds showed mild activity against *C. albicans.* Positive controls showed good inhibition zones against the tested bacteria and fungi,
however, no inhibitory effect was observed in case of negative control against any of the test organisms as shown in Table 4 and 5.

On the basis of maximum inhibitory zones shown against Gram positive bacteria, the compound 200a was found to be most effective against B. subtilis and S. aureus with zones of inhibition of 18.6 mm and 17.3 mm, respectively. In antifungal evaluation, compound 200a was found to be most potent against C. albicans with zone of inhibition of 14.3 mm (Table 4).

Among the series, the MIC value of various tested compounds were lie between 64 and 256 µg/mL against bacteria. Compound 200a was found more active as it exhibited the lowest MIC of 64 µg/mL against S. aureus and B. subtilis and lowest MIC of 128 µg/mL against C. albicans (Table 5).

**Table 5.** Minimum inhibitory concentration (MIC) (in µg/mL) of compounds by using agar well diffusion method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>199a</td>
<td>&gt;256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>199b</td>
<td>&gt;256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>199c</td>
<td>&gt;256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>199d</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>Nt</td>
</tr>
<tr>
<td>199e</td>
<td>256</td>
<td>256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>199f</td>
<td>256</td>
<td>256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>199g</td>
<td>256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>200a</td>
<td>65</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>200b</td>
<td>256</td>
<td>128</td>
<td>&gt;256</td>
</tr>
<tr>
<td>200c</td>
<td>128</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>200d</td>
<td>128</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>200e</td>
<td>&gt;256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>200f</td>
<td>128</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>200g</td>
<td>256</td>
<td>256</td>
<td>Nt</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25</td>
<td>6.25</td>
<td>Nt</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>Nt</td>
<td>Nt</td>
<td>128</td>
</tr>
</tbody>
</table>
2.3.2c Structure-activity relationship (SAR) studies

From the present investigation, some generalizations came into account as shown in Figure 86.

1. The compounds of the series were found selectively against Gram-positive bacteria instead of Gram-negative.

2. Among the series, 200a emerged as an excellent antimicrobial agent against S.aureus, B. subtilis and C.albican.

3. Change in functionality from carbonyl group into hydroxyl led to increase in antibacterial potential.

4. Among all the synthesis compounds, the antioxidant activity of compound 199a was found more active than their 4-substituted and reduced hydroxyl derivatives.
Fig. 71 $^1$H NMR spectrum of compound 199b.
Fig. 73 $^{13}$C NMR spectrum of compound 199b.
Fig. 74 HSQC NMR spectrum of compound 199b.
Fig. 74' Expanded HSQC NMR spectrum of compound 199b.
Fig. 75 HMBC NMR spectrum of compound 199b.
Fig. 75' Expanded HMBC NMR spectrum of compound 199b.
Fig. 77 COSY NMR spectrum of compound 199b.
Fig. 77' Expanded COSY NMR spectrum of compound 199b.
Fig. 78 ROESY NMR spectrum of compound 199b.
Fig. 79 $^1$H NMR spectrum of compound 200b.
Fig. 81 $^{13}$C NMR spectrum of compound 200b.
Fig. 82 HSQC NMR spectrum of compound 200b.
Fig. 82 HSQC NMR spectrum of compound 200b.
Fig. 83 HMBC NMR spectrum of compound 200b.
Fig. 83' Expanded HMBC NMR spectrum of compound 200b.
Fig. 84 COSY NMR spectrum of compound 200b.
Fig. 84' Expanded COSY NMR spectrum of compound 200b.
Fig. 86 ROESY NMR spectrum of compound 200b.
REFERENCES


