CHAPTER-II, SECTION-3

Development of biologically active hydroxymoyl chlorides as potent antifungal agents
2.3.1. Introduction

The recent decades are marked by the increased incidence of fungal diseases and the emergence of several fungi as opportunistic pathogens. These opportunistic fungal pathogens have increased substantially in immunocompromised patients. Furthermore, mortality rates on account of these infections remain unacceptably high: nearly 30% in invasive aspergillosis, 39% in invasive candidiasis, about 70% in fusarium infections and up to 80% in zygomycosis.1-4 Although amphotericin B remains the standard therapy for many invasive or life-threatening mycoses, this polyene drug is associated with significant toxicity, including infusion-related events, such as chills, fever, headache, nausea, vomiting and dose-limiting nephrotoxicity.5 In addition, the clinical efficacy of amphotericin B in some settings (e.g., mold disease such as invasive aspergillosis in severely immunocompromised patients) is suboptimal. However, more recently, there has been a considerable expansion in the number of antifungal drugs. Few major classes of antifungal compounds are currently in clinical use: polyenes,azole derivatives, echinocandins, allylamines,thiocarbamates, fluoropyrimidines, imidazoles, oxime derivatives, etc.6-8 Despite this growing list of antifungal agents, in many cases, treatment of fungal diseases remains unsatisfactory because of toxicity, low efficacy and drug resistance. This situation has led to search for fungicidal agents with a new mode of action and with fewer side effects, which can be administered both orally and parenterally and may overcome the limitations of current antifungal agents.6,9-12

2.3.2. Present work

Even though oximes have been used as pharmacophoric groups for the generation of highly effective anti-microbials, no efforts have been hitherto made to explore the biological potential of their halogenated analogs, i.e., chlorooximes. The current study is an attempt to synthesize and assess particularly the antifungal effects of various chlorooximes on different strains of fungi such as Candida albicans, C. parapsilosis, C. glabrata, C. krusei, Aspergillus fumigatus, A. flavus and A. niger to explore their therapeutic potential. Chemically, chlorooximes are important intermediates for the synthesis of nitrile oxides which in turn are used in a number of chemical reactions such as dipolar cycloaddition reactions and lead to the synthesis of a variety of heterocycles like isoxazoles, isoxazolines, etc. In the present section,
we report the synthesis of a focused library of chlorooximes and their antifungal activity including minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) against standardized ATCC isolates. A brief discussion about the structure-activity relationship (SAR) of the investigated oxime derivatives in comparison to the corresponding parent oximes is also presented.

Scheme 1: Synthesis of oximes and chloroximes

The oximes and chlorooximes were synthesized as per the literature procedure (Scheme 1). To the neutralized solution of hydroxylamine hydrochloride (NH$_2$OH.HCl), aldehyde (1) was added and the reaction mixture stirred for 1 h at ambient temperature. After completion of the reaction (monitored by TLC), excess of water was added to the reaction mixture and organic compound extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and concentrated under vacuum to afford pure oxime (mixture of syn and anti) in 99% yield. Oximes (2), when treated with N-chlorosuccinimide in DMF led to the synthesis of corresponding chlorooximes (3) in good yields (>85%) (Table 1).

2.3.3. Experimental Section

2.3.3.1. Synthesis

The experimental details pertaining to the synthesis of aimed compounds in this study are given as under:

Typical procedure for the synthesis of 2,3-dimethoxybenzaldoxime (2q):
In a typical procedure, NH$_2$OH.HCl (0.50 g, 7.22 mmol) was dissolved in water and neutralized with NaOH. To the above neutralized solution, 2,3-dimethoxybenzaldehyde (1.00 g, 6.02 mmol) was added and the reaction mixture stirred for 1 h at ambient temperature. After completion of the reaction (monitored by TLC), excess of water was added to the reaction mixture and the organic compound extracted with EtOAc (2 × 50 mL). The combined organic layers were
dried over anhydrous Na₂SO₄ and concentrated under vacuum to afford pure oxime (mixture of syn and anti) in 99% yield.

**Typical procedure for the synthesis of 2,3-dimethoxy phenyl hydroxymoyl chloride (3q):**

2,3-Dimethoxybenzaldoxime (1.00 g, 5.52 mmol) was dissolved in DMF (20 mL). N-chlorosuccinimide (0.95 g, 7.18 mmol) was added to the above solution and the reaction mixture stirred for 8-10 h. After completion of the reaction (monitored by TLC), excess of water was added to the reaction mixture and organic compound extracted with Et₂O (3 × 50 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under vacuum to afford pure chlorooxime (syn and anti). The chlorooxime so formed was subjected to column chromatography [silica gel 230-400 mesh as stationary phase, hexane:EtOAc; (7:3) as mobile phase] and both geometrical isomers were isolated in pure form.
Table 1: Synthesis of various oximes and their corresponding chlorooximes
2.3.3.2. Spectral data

Phenylhydroxymoyl chloride (3a):

\[
\begin{align*}
\text{White solid; mp:} & \quad 50-51 \degree C. \\
^1\text{H NMR (200 MHz, CDCl}_3\text{):} & \quad \delta 7.42 (m, 3H), 7.85 (m, 2H). \\
\text{IR (KBr, cm}^{-1}\text{):} & \quad 691, 763, 935, 1101, 1181, 1236, 1387, 1446, 1493, 1654, 1706, 2927, 3061, 3210. \\
\text{Mass (ESI-MS):} & \quad 155.58 (M^+). \\
\text{C, H, N analysis for} & \quad \text{C}_7\text{H}_6\text{ClNO:} \quad \text{Calculated C, 54.04; H, 3.89; N, 9.00. Found C,} \\
& \quad \text{53.98; H, 4.00; N, 9.11.}
\end{align*}
\]

N-hydroxythiophene-2-carbimidoyl chloride (3b):

\[
\begin{align*}
\text{Brown solid; mp:} & \quad 102-103 \degree C. \\
^1\text{H NMR (200 MHz, CDCl}_3\text{):} & \quad \delta 2.13 (s, 1H), 6.88 (m, 2H), 7.05 (m, 1H). \\
\text{IR (KBr, cm}^{-1}\text{):} & \quad 710, 800, 836, 857, 877, 993, 1237, 1422, 1597, 1649, 2923, 3106, 3321. \\
\text{Mass (ESI-MS):} & \quad 184.61 (M^+ + \text{Na}). \\
\text{C, H, N analysis for} & \quad \text{C}_5\text{H}_4\text{ClNOS:} \quad \text{Calculated C, 37.16; H, 2.49; N, 8.67. Found C,} \\
& \quad \text{36.97; H, 2.44; N, 8.99.}
\end{align*}
\]

2-Nitro-phenylhydroxymoyl chloride (3c):

\[
\begin{align*}
\text{Yellow solid; mp:} & \quad 97-98 \degree C. \\
^1\text{H NMR (200 MHz, CDCl}_3\text{):} & \quad \delta 2.01 (s, 1H), 7.66 (m, 2H), 7.90 (d, 1H, J = 7.62 \text{ Hz}), 8.20 (d, 1H, J = 7.29 \text{ Hz}).
\end{align*}
\]
IR (KBr, cm\(^{-1}\)): 639, 755, 919, 1183, 1295, 1351, 1704, 1773, 2854, 2926, 3230, 3446.

Mass (ESI-MS): 201 (M\(^{+}\) + H).

C, H, N analysis for C\(_7\)H\(_5\)ClN\(_2\)O\(_3\):

Calculated C, 41.92; H, 2.51; N, 13.97. Found C, 41.96; H, 2.54; N, 13.94.

\textbf{N-hydroxynicotinimidoyl chloride (3d):}

\[
\text{\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{OH}
\end{array}}
\]

White solid; mp: 143-145 \(^{\circ}\)C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 2.08\) (s, 1H), 7.68 (m, 1H), 8.23 (d, 1H, \(J = 7.78\) Hz), 8.78 (d, 1H, \(J = 8.12\) Hz), 8.96 (s, 1H).

IR (KBr, cm\(^{-1}\)): 698, 710, 796, 807, 963, 1187, 1337, 1482, 1517, 1666, 2828, 3126, 3228.

Mass (ESI-MS): 195 (M\(^{+}\) + K).

C, H, N analysis for C\(_6\)H\(_5\)ClN\(_2\)O:

Calculated C, 46.03; H, 3.22; N, 17.89. Found C, 46.08; H, 3.28; N, 17.84.

\textbf{4-Cyano-phenylhydroxymoyl chloride (3e):}

\[
\text{\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{OH}
\end{array}}
\]

White solid; mp: 151-152 \(^{\circ}\)C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 2.02\) (s, 1H), 7.56 (d, 2H, \(J = 7.24\) Hz), 7.78 (d, 2H, \(J = 7.63\) Hz).

IR (KBr, cm\(^{-1}\)): 639, 664, 758, 1100, 1180, 1244, 1292, 1361, 1388, 1456, 1660, 1708, 1773, 2232, 2302, 2927, 3435.

Mass (ESI-MS): 205 (M\(^{+}\) + Na).

C, H, N analysis for C\(_8\)H\(_5\)ClN\(_2\)O:

Calculated C, 53.21; H, 2.79; N, 15.51. Found C, 53.28; H, 2.73; N, 15.56.
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*N-hydroxy-1H-indole-3-carbimidoyl chloride (3f):*

![Chemical structure](image)

White solid; mp: 180-181 °C.

$^1$H NMR (200 MHz, CDCl$_3$): \( \delta \) 2.01 (s, 1H), 7.12-7.31 (m, 2H), 7.38 (s, 1H), 7.43 (d, 1H, \( J = 7.46 \) Hz), 7.62 (d, 1H, \( J = 7.57 \) Hz), 10.21 (s, 1H).

IR (KBr, cm$^{-1}$): 684, 729, 801, 966, 1102, 1254, 1327, 1491, 1523, 1665, 2185, 2264, 2934, 3301, 3422.

Mass (ESI-MS): 193 (M$^+$ - H).


*4-Dimethylamino-phenylhydroxymoyl chloride (3g):*

Gummy liquid

$^1$H NMR (200 MHz, CDCl$_3$): \( \delta \) 2.01 (s, 1H), 3.12 (s, 6H), 7.43 (d, 2H, \( J = 7.46 \) Hz), 7.62 (d, 2H, \( J = 7.57 \) Hz).

IR (CHCl$_3$, cm$^{-1}$): 641, 713, 806, 1020, 1179, 1242, 1294, 1402, 1703, 1772, 2361, 2927, 3387.

Mass (ESI-MS): 199 (M$^+$).

C, H, N analysis for C$_9$H$_{11}$ClN$_2$O: Calculated C, 54.42; H, 5.58; N, 14.10. Found C, 54.49; H, 5.50; N, 14.08.
4-Fluoro-phenylhydroxymoyl chloride (3h):

White solid; mp: 72-73 °C.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 2.02 (s, 1H), 7.02 (d, 2H, $J = 7.28$ Hz), 7.63 (d, 2H, $J = 7.58$ Hz).

IR (KBr, cm$^{-1}$): 590, 664, 769, 840, 935, 981, 1055, 1159, 1237, 1294, 1411, 1430, 1506, 1603, 1682, 1765, 3076, 3365.

Mass (ESI-MS): 196 (M$^+$ + Na).

C, H, N analysis for C$_7$H$_5$ClFNO: Calculated C, 48.44; H, 2.90; N, 8.07. Found C, 48.48; H, 2.92; N, 8.04.

4-Chloro-phenylhydroxymoyl chloride (3i):

White solid; mp: 76-77 °C.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 1.70 (s, 1H), 7.38 (d, 2H, $J = 8.74$ Hz), 7.80 (d, 2H, $J = 8.77$ Hz).

IR (KBr, cm$^{-1}$): 665, 828, 936, 1014, 1093, 1245, 1401, 1488, 1595, 1650, 2852, 2924, 3292.

Mass (ESI-MS): 191.03 (M$^+$ + H).

C, H, N analysis for C$_7$H$_5$Cl$_2$NO: Calculated C, 44.24; H, 2.65; N, 7.37. Found C, 44.39; H, 2.85; N, 7.12.
3-Nitro-phenylhydroxymoyl chloride (3j):

![Chemical structure]

Yellow solid; mp: 99-100 °C.

\[^1\text{H NMR (200 MHz, CDCl}_3\text{:} \delta 2.04 \text{ (s, 1H), 7.62 (m, 1H), 8.00 (d, 1H, } J = 7.28 \text{ Hz), 8.20 (d, 1H, } J = 7.65 \text{ Hz), 8.60 (s, 1H).}]

IR (KBr, cm\(^{-1}\)):

641, 758, 1017, 1183, 1352, 1427, 1532, 1706, 1744, 2304, 2855, 2927, 3244.

Mass (ESI-MS):

201 (M\(^+\) + H).

C, H, N analysis for C\(_7\)H\(_5\)ClN\(_2\)O\(_3\):  Calculated C, 41.92; H, 2.51; N, 13.97. Found C, 41.96; H, 2.54; N, 13.94.

2,6-Difluoro-phenylhydroxymoyl chloride (3k):

![Chemical structure]

White solid; mp: 104-106 °C.

\[^1\text{H NMR (200 MHz, CDCl}_3\text{:} \delta 2.03 \text{ (s, 1H), 6.82 (d, 2H, } J = 7.29 \text{ Hz), 7.31 (m, 1H).}]

IR (KBr, cm\(^{-1}\)):

644, 751, 964, 1105, 1190, 1253, 1390, 1438, 1661, 1710, 2307, 2935, 3440.

Mass (ESI-MS):

192 (M\(^+\) + H).

C, H, N analysis for C\(_7\)H\(_4\)ClF\(_2\)NO:  Calculated C, 43.89; H, 2.10; N, 7.31. Found C, 43.81; H, 2.14; N, 7.37.

N-hydroxybutyrimidoyl chloride (3l):

![Chemical structure]

White solid; mp: 50-52 °C.
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\(^1\)H NMR (200 MHz, CDCl\(_3\)):  \(\delta 0.92\) (t, 3H, \(J = 2.84\) Hz), 1.33 (t, 2H, \(J = 3.43\) Hz), 1.58 (m, 2H), 2.03 (s, 1H).

IR (KBr, cm\(^{-1}\)):  712, 945, 1034, 1209, 1312, 1754, 2992, 3004, 3296, 3310.

Mass (ESI-MS):  120 (M\(^+\) - H).

C, H, N analysis for 
\(\text{C}_4\text{H}_8\text{ClNO}\):  Calculated C, 39.52; H, 6.63; N, 11.52. Found C, 39.56; H, 6.67; N, 11.59.

4-Methyl-phenylhydroxymoyl chloride (3m):

\[
\text{Cl} \quad \text{H}_2\text{C} \quad \text{N} \quad \text{OH} \\
\text{H}_2\text{C} \quad \text{N} \quad \text{OH}
\]

Off white solid; mp:  71-72 °C.
\(^1\)H NMR (200 MHz, CDCl\(_3\)):  \(\delta 2.24\) (s, 1H), 2.40 (s, 3H), 7.24 (d, 2H, \(J = 8.67\) Hz), 7.42 (d, 2H, \(J = 8.68\) Hz).

IR (KBr, cm\(^{-1}\)):  663, 832, 896, 1019, 1097, 1387, 1558, 1655, 2341, 2360, 2924, 3384.

Mass (ESI-MS):  169.62 (M\(^+\)).

C, H, N analysis for 
\(\text{C}_8\text{H}_8\text{ClNO}\):  Calculated C, 56.65; H, 4.75; N, 8.26. Found C, 56.53; H, 4.99; N, 8.32.

2,4-Dimethoxy-phenylhydroxymoyl chloride (3n):

\[
\text{Cl} \quad \text{H}_3\text{CO} \quad \text{N} \quad \text{OH} \\
\text{H}_3\text{CO} \quad \text{N} \quad \text{OH}
\]

White solid; mp:  153-154 °C.
\(^1\)H NMR (200 MHz, CDCl\(_3\)):  \(\delta 3.85\) (s, 3H), 4.02 (s, 3H), 6.7 (s, 1H), 7.11-7.14 (m, 2H).

IR (KBr, cm\(^{-1}\)):  692, 781, 839, 928, 1088, 1143, 1213, 1333, 1438, 1482, 1544, 2149, 2219, 2962, 3143, 3382.

Mass (ESI-MS):  215.65 (M\(^+\)).

C, H, N analysis for
C₉H₁₀ClNO₃: Calculated C, 50.13; H, 4.67; N, 6.50. Found C, 50.19; H, 4.69; N, 6.52.

4-Methoxy-phenylhydroxymoyl chloride (3o):

![Structure of 4-Methoxy-phenylhydroxymoyl chloride]

White solid; mp: 89-90°C.

H NMR (200 MHz, CDCl₃):  δ 2.05 (s, 1H), 3.82 (s, 3H), 6.87 (d, 2H, J = 7.22 Hz), 7.62 (d, 2H, J = 7.56 Hz).

IR (KBr, cm⁻¹): 664, 834, 935, 1027, 1175, 1256, 1304, 1462, 1511, 1606, 1661, 2840, 2964, 3195.

Mass (ESI-MS): 224.65 (M⁺ + K).


3-Methyl-phenylhydroxymoyl chloride (3p):

![Structure of 3-Methyl-phenylhydroxymoyl chloride]

Off white solid; mp: 49-50°C.

H NMR (200 MHz, CDCl₃):  δ 2.01 (s, 1H), 2.39 (s, 3H), 7.17-7.24 (m, 3H), 7.41 (s, 1H).

IR (KBr, cm⁻¹): 686, 702, 873, 903, 1048, 1108, 1329, 1559, 1661, 2298, 2392, 2937, 3321.

Mass (ESI-MS): 169.62 (M⁺).

2,3-Dimethoxy-phenylhydroxymoyl chloride (3q):

White solid; mp: 112-113 °C.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 3.86 (s, 3H), 3.88 (s, 3H), 6.93 (d, 2H, $J = 8.06$ Hz), 7.35 (d, 1H, $J = 7.78$ Hz)

IR (KBr, cm$^{-1}$): 738, 768, 972, 984, 1004, 1173, 1221, 1320, 1424, 1477, 1578, 1998, 2975, 3020, 3252.

Mass (ESI-MS): 215.65 (M$^+$).

C, H, N analysis for C$_9$H$_{10}$ClNO$_3$: Calculated C, 50.13; H, 4.67; N, 6.50. Found C, 50.25; H, 4.72; N, 6.66.

2,3-Dimethoxy-phenylhydroxymoyl chloride (3q) (syn):

White solid; mp: 108-109 °C.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 3.93 (s, 3H), 4.02 (s, 3H), 6.70 (d, 2H, $J = 8.06$ Hz), 7.10 (d, 1H, $J = 8.01$ Hz).

IR (KBr, cm$^{-1}$): 669, 786, 858, 931, 1005, 1018, 1055, 1265, 1297, 1333, 1419, 1431, 1471, 1573, 2309, 2358, 2923, 3081, 3405.

Mass (ESI-MS): 215.65 (M$^+$).

C, H, N analysis for C$_9$H$_{10}$ClNO$_3$: Calculated C, 50.13; H, 4.67; N, 6.50. Found C, 50.00; H, 4.78; N, 6.39.
2,3-Dimethoxy-phenylhydroxymoyl chloride (3q) (anti):

White solid; mp: 90-91 °C.

$^1$H NMR (200 MHz, CDCl$_3$):
δ 3.87 (s, 3H), 3.98 (s, 3H), 6.93 (d, 2H, $J = 8.93$ Hz), 7.12 (d, 1H, $J = 8.94$ Hz).

IR (KBr, cm$^{-1}$):
617, 675, 808, 836, 901, 1006, 1045, 1095, 1232, 1274, 1339, 1420, 1477, 1574, 2310, 2359, 2924, 3081, 3355.

Mass (ESI-MS): 216.65 (M$^+$ + H).

C, H, N analysis for C$_9$H$_{10}$ClNO$_3$:
Calculated C, 50.13; H, 4.67; N, 6.50. Found C, 50.28; H, 4.97; N, 6.44.

N-hydroxybenzo[d][1,3]dioxole-5-carbimidoyl chloride (3r):

White solid; mp: 126-127 °C.

$^1$H NMR (200 MHz, CDCl$_3$):
δ 5.92 (s, 2H), 6.54 (d, 1H, $J = 7.18$ Hz), 7.12 (m, 2H).

IR (KBr, cm$^{-1}$):
687, 779, 987, 1102, 1192, 1265, 1301, 1426, 1513, 1644, 1734, 2922, 3097, 3318.

Mass (ESI-MS): 199.58 (M$^+$).

C, H, N analysis for C$_9$H$_6$ClNO$_3$:
Calculated C, 48.14; H, 3.03; N, 7.02. Found C, 48.18; H, 3.09; N, 7.04.

N-hydroxy-1-naphthimidoyl chloride (3s):

White solid; mp: 97-98 °C.
1H NMR (200 MHz, CDCl3): δ 1.98 (s, 1H), 7.32 (d, 2H, J = 7.02 Hz), 7.58-7.69 (m, 3H), 7.99 (d, 1H, J = 7.67 Hz), 8.04 (d, 1H, J = 7.34 Hz).

IR (KBr, cm⁻¹): 658, 775, 803, 916, 988, 1096, 1176, 1238, 1255, 1387, 1410, 1436, 1509, 1659, 2926, 3057, 3196.

Mass (ESI-MS): 229 (M⁺ + Na).

C, H, N analysis for C₁₁H₈CINO: Calculated C, 64.25; H, 3.92; N, 6.81. Found C, 64.20; H, 3.95; N, 6.84.

N-hydroxy-2-naphthimidoyl chloride (3t):

Gummy liquid

1H NMR (200 MHz, CDCl₃): δ 1.92 (s, 1H), 7.54 (m, 2H), 7.80-7.95 (m, 3H), 8.00 (d, 1H, J = 8.69 Hz), 8.34 (s, 1H).

IR (CHCl₃, cm⁻¹): 474, 581, 616, 657, 750, 820, 861, 1095, 1125, 1185, 1271, 1404, 1503, 1602, 1630, 1701, 1705, 2298, 2347, 3144.

Mass (ESI-MS): 229 (M⁺ + Na).

C, H, N analysis for C₁₁H₈CINO: Calculated C, 64.25; H, 3.92; N, 6.81. Found C, 64.18; H, 3.99; N, 6.65.

3,4-Dihydroxy-phenylhydroxymoyl chloride (3u):

Gummy liquid

1H NMR (200 MHz, CDCl₃): δ 1.98 (s, 1H), 7.32 (d, 1H, J = 7.02 Hz), 7.99 (d, 1H, J = 7.67 Hz), 8.04 (s, 1H).

IR (CHCl₃, cm⁻¹): 640, 665, 817, 1001, 1104, 1182, 1251, 1294, 1406, 1653, 1705, 2298, 2347, 3144.
Mass (ESI-MS): \[188 (M^+ + H)\].

C, H, N analysis for C\(_7\)H\(_6\)ClNO\(_3\):
Calculated C, 44.82; H, 3.22; N, 7.47. Found C, 44.89; H, 3.20; N, 7.44.

\(N\)-hydroxyanthracene-1-carbimidoyl chloride (3v):

Yellow solid; mp: 114-115 °C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)):
\(\delta 1.98 (s, 1H), 7.51-7.70 (m, 4H), 8.04 (d, 2H, J = 8.36 \text{ Hz}), 8.27 (d, 2H, J = 8.71 \text{ Hz}), 8.54 (s, 1H)\).

IR (KBr, cm\(^{-1}\)):
658, 775, 803, 916, 988, 1096, 1176, 1238, 1255, 1387, 1410, 1436, 1509, 1659, 2926, 3057, 3196.

Mass (ESI-MS):
\[278 (M^+ + Na)\].

C, H, N analysis for C\(_{15}\)H\(_{10}\)ClNO:
Calculated C, 70.46; H, 3.94; N, 5.48. Found C, 70.54; H, 3.88; N, 5.55.

\subsection*{2.3.3.3. Biological experiments}

All the synthesized compounds (Table 1) were screened for their anti-fungal activity against seven fungal strains viz., Candida albicans, Candida parapsilosis, Candida glabrata, Candida krusei, Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger, using microdilution technique.

\section*{Material and Methods}

Antifungal activity of all the compounds was performed using microdilution method (NCCLS M27 A, NCCLS M38 P) against four yeast strains (Candida albicans ATCC 90028, Candida parapsilosis ATCC 22019, Candida glabrata ATCC 90030, and Candida krusei ATCC 6258) and three filamentous fungi (Aspergillus fumigatus LSI-II, Aspergillus niger ATCC 16404, Aspergillus flavus MTCC 2799). The ATCC cultures used for this study were purchased from American Type Culture Collection, Manassas, VA 20108 USA. RPMI supplemented with 0.165 M MOPS was used as test media. The MIC (minimum inhibitory concentration) was determined by serial 2-fold dilution of the test compound in the above-mentioned media in 100 µL volume in

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a 96 well U bottom microtitre plate. Yeast inoculums were prepared by growing isolates on Sabouraud dextrose agar plates overnight at 37 °C. The isolated colonies were picked up and suspensions were prepared in sterile normal saline with 0.05% (vol/vol) Tween 80 (NST). The density of these suspensions was adjusted to 1 McFarland (1-5 × 10⁶ CFU/mL), further diluted to 1:50 in NST and 1:20 in RPMI 1640 media with 0.165 M MOPS to get 2 times the final inoculum (1-5 × 10³ CFU/mL). For filamentous fungi, the inoculums were prepared from the spores of the cultures, which were sporulated on potato dextrose agar (PDA) after incubation at 28 °C for 7 days. The density of the spore suspension was adjusted to an optical density of 0.09 to 0.11. These suspensions were diluted 1:50 in RPMI 1640 media with 0.165 M MOPS to get the final inoculum (0.4 × 10⁴ to 5 × 10⁴ CFU/mL). 100 μL of this 2 × inoculum of yeast and fungi was added to each well of the microtitre plate. The plates were incubated at 37 °C for 48 h. The plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC. The MFC was determined by spotting 10 μL volume on Sabouraud dextrose agar plate from the wells showing no visible growth. The plates were incubated at 37 °C for 48 h.¹⁴,¹⁵ Minimum concentration of compound showing absence of growth was recorded as MFC. Amphotericin-B being the drug of choice for inhibiting cell growth was taken as a standard.

**2.3.4. Results**

The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of the oximes and chlorooximes are presented in table 2.
## Test organisms

<table>
<thead>
<tr>
<th>Entry</th>
<th>Yeast</th>
<th>Filamentous Fungi</th>
</tr>
</thead>
<tbody>
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Table 2: The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of oximes, chlorooximes and the control drug. MIC and MFC are expressed in \( \mu g/mL \).

Table 3: The comparative MIC and MFC values for each isomer of compound 3q. MIC and MFC are expressed in \( \mu g/mL \).
2.3.5. Discussion

Antifungal activity and structure activity relationship studies

From the recorded observations, it is evident that most of the chlorooximes show interesting antifungal activity (MICs \( \leq 32 \mu g/mL \)) and comparative to oximes, their MICs are more attractive. Among the investigated derivatives, compounds 3a, 3b, 3i, 3m, 3q, 3r, 3s, 3t and 3v showed significant activity against *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *A. fumigatus*, *A. flavus* and *A. niger* with MICs in single digits and compound 3q was the most active. This compound was very potent against all the *Candida* species (MIC 0.5 \( \mu g/mL \)). It was also active against filamentous fungi with MIC range of 2-4 \( \mu g/mL \). This series of compounds was fungicidal in nature as is evident from the MFC results and the compound 3q was found to be the most active (MFC 0.5 \( \mu g/mL \)). SAR studies on the investigated compounds reveal that oximes are less potent than their corresponding chlorooxime derivatives. Among chlorooximes, compound 3q was found to be active against all the strains of *Candida* and *Aspergillus* species. Even though a variety of chlorooximes derived from phenyl, substituted phenyl and heteroaromatic oximes exhibited potent antifungal activity, presence of electron donating and electron withdrawing groups on aromatic nucleus was found to be ineffective in changing their MIC and MFC values appreciably. Similarly, it was observed that bulky aromatic rings like naphthyl and anthracyl oximes did not have profound effect on the antifungal activity. Chlorooximes derived from aliphatic oximes showed lower activity in comparison to their aromatic counterparts. Since these compounds exist in two isomeric forms *i.e.*, syn and anti, the need to examine the antifungal activity of each isomeric form was felt. Thus, compound 3q being the most potent antifungal derivative, was subjected to column chromatography (silica gel 230-400 mesh as stationary phase, hexane/ethylacetate as mobile phase) and both geometrical isomers were isolated in pure form. The isomers were identified on the basis of their coupling constant values, syn-isomer having coupling constant values of 8.01 Hz and 8.06 Hz whereas anti-isomer having coupling constant values of 8.93 Hz and 8.94 Hz. NO stretching values for syn and anti were found to be 931.46 cm\(^{-1}\) and 901.59 cm\(^{-1}\) respectively. The distinction between syn and anti could also be made on the basis of polarity (syn being more polar than anti) and their respective melting point differences (syn-isomer having...
melting point of 108-109 °C and anti-isomer having melting point of 90-91 °C). Each isomer thus isolated was screened for anti-fungal activity against the above mentioned seven strains and observed results are enlisted in table 3. From these values it is clear that anti-isomer is more potent than syn-isomer.

It is now well established that the zinc and calcium dependent family of proteins called the MMPs (matrix metalloproteinases) which are secreted by fungus such as Candida albicans, hydrolyse the collagen proteins on skin and consequently cause fungal infections under physiological conditions. The involved steps are selectively regulated by endogenous inhibitors and imbalances between the active enzymes and their natural inhibitors lead to the fungal disease. Use of specific enzyme inhibitors to redress this balance as a potential cure of such infections has led to intensive research focused on the design, synthesis\textsuperscript{16-18} and molecular deciphering of low molecular mass inhibitors of this family of proteins. Derivatives such as oximes and hydrazides, possess selective chelating or binding properties with the zinc active-site of MMPs. Hence such small molecule MMP inhibitors can act either as competitive substrates or distort the geometry of one of zinc centers in MMPs by binding with such zinc cations in the form of a five or six-member ring with one or two double bonds, respectively, in a bidentate structure form. After distorting the geometry of such zinc cations, these MMP inhibitors appear to move away from this "deactivated" active-site and go to the next active-site to deactivate it.\textsuperscript{19} Since chloroximes and oximes are strong ligands for zinc binding, we envisage a similar mechanism for their antifungal action.

2.3.6. Conclusion

In conclusion hydroxymoyl chlorides as novel antifungal agents have been presented. The results obtained here would provide a useful clue for the design and development of new antifungal agents.
2.3.7. References


