Synthesis and Analysis of Antimicrobial Properties of Isoxazoline

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I Abstract: A simple procedure for the synthesis of isoxazole derivatives was developed. Cyclocondensation reaction of chalcones with hydroxylamine hydrochloride catalyzed by an acid produced isoxazoles. Chalcones were synthesized by the condensation product of acetophenone in combination with aromatic aldehydes in presence of strong base. The chalcone synthesized by base catalyzed condensation of 3-acetyl-6-methyl-2H-pyran-2,4-(3H) dione (DHA) with different aromatic aldehydes in good yields. These chalcones were used for synthesis of derivatives i.e. isoxazole. The synthesized new compounds were characterized by spectral studies i.e. IR, 1H NMR and mass spectral analysis and elemental analysis; before being were screened in vitro for their antimicrobial susceptibilities against different bacteria and fungi species. The derivatives were further used for the estimation of its biological properties. From the study it was found that the synthesized compounds are efficient for further researchwork.

II Keywords: Dehydroacetic acid (DHA), Chalcone, 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyrones, IR, 1H NMR, Antibacterial activity, Antifungal activity, Isoxazoline.

III INTRODUCTION

Isoxazole is a five member heterocyclic compounds containing N and O atoms adjacent to each other’s. The dihydric derivative of isoxazoline are also called as isoxazole the compound containing N functionality (-C=N-O-) undergo cyclo addition reaction with an α, β-unsaturated carbonyl compound or with an alkane to produce isoxazoline having an –N-O- moiety, O being next to N rises the barrier to N inversion to such extent that a temperature lower than the ambient, individual can be identified by 1H NMR spectroscopy1-2.

Synthesis of isoxazoline derivatives has been a subject of consistent interest because of the wide applications of such heterocyclic in pharmaceutical as well as agrochemical industry3. Numerous compounds containing isoxazole, pyrazole and pyrimidine moiety have been reported as active hypoglycemic, antidiabetic, antipyretics, analgesics, antiinflammatory, antiviral, antiallergic, anticancer agent, antirepressant and antimicrobial agents which include antibacterial sulfonamides, semisynthetic penicillins and cephalosporines4-6. These discoveries led the scientist to attempt the synthesis of isoxazoline, pyrazole and pyrimidine derivatives from the chalcones made from aromatic aldehydes and aromatic ketones and to evaluate the synthesized compounds against antimicrobial, antifungal and antitubercular activity7-10.

The reactive intermediate chalcones involved in their synthesis also exhibit wide range of biological activities11. These properties were used in the research to find some novel compounds for pharmaceutical and agricultural industry.
IV METHODOLOGY

Synthesis of Substituted isoxazoline

A mixture of DHA chalcone (0.001mol) and hydroxy amine hydrochloride (0.0015mol) and sodium acetate (0.002mol) were refluxed for 6-7hr in 15ml ethanol. After completion of the reaction (checked by TLC), the reaction mixture was cooled and poured on ice cold water. The separated solid product was filtered, washed with cold water, dried and further recrystallized from ethyl alcohol. The structures of isoxazolines were confirmed by spectral analysis (IR, $^1$H NMR, Mass and Elemental analysis)\textsuperscript{12-13}.

Analytical Characterizations

The IR spectra of compound were recorded on FT-IR spectrometer. $^1$H NMR AVANCE 500 MHz (CDCl$_3$) instrument using TMS as an internal standard (chemical shift are given in $\delta$ ppm). The mass spectra recorded on SHIMADZU spectrometer.

IR Spectra

The IR spectra showed characteristics absorption band at 1610-1640cm$^{-1}$ due to the C=O stretching. A broad peak showed in between 3000-3500cm$^{-1}$ is due to the hydroxyl group in synthesized compounds.

$^1$H NMR spectra

$^1$H NMR of isoxazoline showed following type of peak which confirms the formation of product $\delta$ 3.0-3.8 (dd, 2H, CH$_2$) \textit{i.e.} H$_x$ and H$_y$ of isoxazoline ring. $\delta$ 5.0-5.4 (1H, CH) \textit{i.e.} H$_z$ of isoxazoline ring. $\delta$ 14.2-16.0 (s, 1H, OH) while the ring peak at $\delta$ 2.15-2.8 (s, 3H, Ar-CH$_3$) all such peaks are observed.

Mass Spectra

Mass Spectra of representative compounds confirmed the molecular formula weight of respective compounds.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Synthesis of 3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(Substituted aryl)-4,5-dihydro-2-isoxazoline (MBO-I-MBO-V) (MBO-I-MBO-V) }
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Sr. No. & Reactant & Product & Yield (\%) & M.P. (\degree C) & Time (hr) \\
\hline
1 & \includegraphics[width=0.2\textwidth]{table1_1.png} & \includegraphics[width=0.2\textwidth]{table1_2.png} & 72 & 222 & 6 \\
\hline
2 & \includegraphics[width=0.2\textwidth]{table1_3.png} & \includegraphics[width=0.2\textwidth]{table1_4.png} & 70 & 190 & 7 \\
\hline
3 & \includegraphics[width=0.2\textwidth]{table1_5.png} & \includegraphics[width=0.2\textwidth]{table1_6.png} & 72 & 215 & 8 \\
\hline
\end{tabular}
\caption{Physical data of 3-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-5-(Substituted aryl)-4,5-dihydro-2-isoxazoline (MBO-I-MBO-V)}
\end{table}
Spectroscopic data

**Compound No. MBO-I**

3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(3-methoxyphenyl)-4,5-dihydro-2-Isoxazoline

**IR(KBr,cm⁻¹):** 3422 (OH str.), 2970 (C-H str. of CH₃), 1720 (C=O lactone), 1622 (C=N str. of Isoxazoline ring), 1230 (C-N str. of Isoxazoline ring), 822 (N-O Str.)

**¹H NMR(CDCl₃,δ/ppm):** δ2.1 (3H, s, CH₃), δ3.4 (1H, dd, CH₂Isoxazoline ring), δ3.9 (1H, dd, CH₂Isoxazoline ring), δ5.4 (1H, t, CH Isoxazoline ring), δ6.0 (1H, s, C₅ DHA), δ6.2-8.0 (4H, m, Ar-H), δ15.2 (1H, s, OH)

**Mass (m/z) :** (M+1) 300

**Compound No. MBO-II**

3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(4-methylphenyl)-4,5-dihydro-2-Isoxazoline

**IR(KBr,cm⁻¹):** 3280 (OH str.), 2932 (C-H str. of CH₃), 1720 (C=O lactone), 1615 (C=N str. of Isoxazoline ring), 1280 (C-N str. of Isoxazoline ring), 845 (N-O Str.)

**¹H NMR(CDCl₃,δ/ppm):** δ2.4 (6H, s, 2XCH₃), δ3.0 (1H, dd, CH₂Isoxazoline ring), δ3.6 (1H, dd, CH₂Isoxazoline ring), δ5.2 (1H, t, CH Isoxazoline ring), δ6.0 (1H, s, C₅ DHA), δ6.8-8.4 (4H, m, Ar-H), δ14.8 (1H, s, OH)

**Mass (m/z) :** (M+1) 286

**Compound No. MBO-III**

3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(4-Bromophenyl)-4,5-dihydro-2-Isoxazoline

**IR(KBr,cm⁻¹):** 3400 (OH str.), 2925 (C-H str. of CH₃), 1725 (C=O lactone), 1622 (C=N str. of Isoxazoline ring), 1265 (C-N str. of Isoxazoline ring), 852 (N-O Str.)

**¹H NMR(CDCl₃,δ/ppm):** δ2.2 (3H, s, CH₃), δ3.6 (1H, dd, CH₂Isoxazoline ring), δ4.0 (1H, dd, CH₂Isoxazoline ring), δ4.8 (1H, t, CH Isoxazoline ring), δ5.9 (1H, s, C₅ DHA), δ6.0-8.0 (4H, m, Ar-H), δ16.0 (1H, s, OH)

**Mass (m/z) :** (M+) 350, (M+2)352

**Compound No. MBO-IV**

3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(4-Chlorophenyl)-4,5-dihydro-2-Isoxazoline

**IR(KBr,cm⁻¹):** 3300 (OH str.), 2970 (C-H str. of CH₃), 1720 (C=O lactone), 1630 (C=N str. of Isoxazoline ring), 1232 (C-N str. of Isoxazoline ring)

**¹H NMR(CDCl₃,δ/ppm):** δ2.4 (3H, s, CH₃), δ3.8 (1H, dd, CH₂Isoxazoline ring), δ4.2 (1H, dd, CH₂Isoxazoline ring), δ5.8 (1H, t, CH Isoxazoline ring), δ6.0 (1H, s, C₅ DHA), δ6.2-8.2 (4H, m, Ar-H), δ15.8 (1H, s, OH)

**Mass (m/z) :** (M+1) 307

**Compound No. MBO-V**

3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(3-Chlorophenyl)-4,5-dihydro-2-Isoxazoline

**IR(KBr,cm⁻¹):** 3320 (OH str.), 2979 (C-H str. of CH₃), 1722 (C=O lactone), 1632 (C=N str. of Isoxazoline ring), 1230 (C-N str. of Isoxazoline ring)

**¹H NMR(CDCl₃,δ/ppm):** δ2.2 (3H, s, CH₃), δ4.0 (1H, dd, CH₂Isoxazoline ring), δ4.6 (1H, dd, CH₂Isoxazoline ring), δ5.8 (1H, t, CH Isoxazoline ring), δ6.0 (1H, s, C₅ DHA), δ6.2-8.0 (4H, m, Ar-H), δ15.8(1H, s, OH)

**Mass (m/z) :** (M+) 380 (M+1) 382

**IV BIOLOGICAL ACTIVITY**

**Preparation of media plates for maintenance of bacteria and fungus**

Nutrient agar (28gm/1000ml) was dissolved in 1lit conical flasks and sterilized in autoclave at 121° C (15 lbs/ sq. inches) for 20 minutes. Potato dextrose agar (39 gm/lit) was dissolved in 1 lit conical flasks and sterilized in autoclave at 121° C (15 lbs/ sq. inches) for 20 minutes. After cooling medium was poured in sterilized petri plate about 25 ml. The media plates were inoculated aseptically with inoculums. The bacterial culture of S. aureus, E. coli, Salmonella typhi were inoculated on nutrient agar medium. The bacterial culture plates were incubated for 24 hrs in Biological oxygen demand (BOD) incubator at 28° C temperature growth of organism. The fungus culture plates were inoculated on potato dextrose agar medium. The fungal culture of Fusarium oxysporum, Candida albicans and Aspergillus flavus plates were incubated at 35-37° C temperature in BOD incubator growth of organism^{14-16}. Antimicrobial activity of compounds by agar well diffusion method

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Prepared media plate which was not inoculated with pathogen was taken and the wells were bored into agar using a sterile 6 mm diameter cork borer, six bore was made in each plate. The culture of the test organism was spread on petri plate with sterilized spreader chalcone derivatives of desired concentration. The test compound (250 μg/mL) was dissolved in dimethyl sulfoxide and loaded on a sterile filter paper disc of 6 mm diameter. The petriplates containing nutrient agar medium were spreaded on PDA. Then the impregnated discs were placed on the surface of inoculated agar medium. With respect to control DMSO is used as a control for testing antimicrobial activity. The positive control was taken as chloramphenicol. The plates were allowed to incubate at room temperature for about 2 hr and then plate were transferred to incubator at 28° C for bacterial culture and 35-37°C for fungal culture. After 3-4 days zone of inhibitions were examined and recorded. The experiments were performed in replication and average diameters of the zones of inhibition were calculated and mentioned in Table 2.

The observation shows that both the substituted chalcone derivatives are positively having antimicrobial property in action. It seems to be bacterial and fungicial activity in them. So, they can be used against different animal and plant pathogens to cure a disease as a protection agent19-20.

| Table 2 Antimicrobial activity of isoxazoline derivatives (MBO-I – MBO-V) |
|-----------------------------------------------|
| **Compound** | **Bacteria** | **Fungi** |
| | **(Zone of Inhibition in mm)** | **(Zone of Inhibition in mm)** |
| | **A** | **B** | **C** | **D** | **E** | **F** |
| **MBO-I** | 23 | 22 | 22 | 20 | 19 | 18 |
| **MBO-II** | 19 | 18 | 23 | 18 | 17 | 17 |
| **MBO-III** | 16 | 17 | 21 | 15 | 14 | 15 |
| **MBO-IV** | 20 | 21 | 23 | 19 | 18 | 16 |
| **MBO-V** | 16 | 15 | 19 | 13 | 14 | 14 |
| **Chloramphenicol (250μg/ml)** | 24 | 25 | 22 | 20 | 22 | 22 |
| **DMSO** | -ve | -ve | -ve | -ve | -ve | -ve |

*Standard. A- Staphylococcus aureus, B- Escherichia coli, C- Salmonella Typhi, D- Fusarium oxysporum, E- Candida albicans, F- Aspergillus flavus.*

**V. RESULT AND DISCUSSION**

A series of novel substituted isoxazoline were synthesized by refluxing a mixture of DHA chalcone (MBC-I-MBC-V), hydroxylamine hydrochloride and sodium acetate in the presence of ethanol, the reaction is completed within 6-7 hr as monitored by TLC providing good to excellent yield 75-80% (MBO-I-MBO-V). All the synthesized substituted isoxazoline derivatives were confirmed on the basis of Spectral analytical data (IR, 1H NMR, Mass and Elemental analysis).

The IR spectra of the isoxazoline derivatives showed disappearance of C=O bond of chalcone-a strong band appears at 1590-1615 cm\(^{-1}\) assigned to C=N because of ring closure. The absorption band 1450-1465 cm\(^{-1}\) due to CH\(_3\) of isoxazoline ring present in the respective compounds.

1H NMR Spectra of some representative compound were recorded which show the characteristic peaks at δ3.0-3.6 (dd, 2H, CH\(_2\) i.e. H\(_A\) and H\(_B\) of isoxazoline ring), 6.52-5.4(t, 1H, CH i.e. H\(_X\) of isoxazoline ring) were observed, all the other aromatic and aliphatic proton were observed at the expected region.

The antimicrobial activities were tested against the bacterial and fungal species as mentioned in methodology, the effective zone of inhibition were observed at each concentrations mentioned in Table 2. All the isoxazoline compounds are having effective antimicrobial properties as shown in Fig 2. The results were compared with standard chloramphenicol and the positive control was taken as DMSO. There was no zone of inhibition in DMSO on microbial growth. Both the organism’s i.e. bacteria and fungi showed maximum zone of inhibition i.e.23 mm and 21 mm, respectively as shown in Table 2.

In conclusion, the synthesized isoxazoline compound possesses a good to moderate biological properties. These compounds will be having application in pharmaceutical, agriculture, medical field for drug development.

**VI. REFERENCES**

[1] Haripara K, Patel S, Joshi A and Paresh H, Indian J Heterocycl Chem., 2004; 13:221.
[2] Wagner E, Becar I and Nowakowska E, Bioorg Med Chem., 2004; 12: 265-272.
[3] Ngami Z, Siti M Haris-Fadzillah, Hasnain Hussain and Kamardzlanon Kamomiddin, World J Chem., 2009; 4(1):09-14.
[4] Vogel A L. Textbook of Practical Organic Chemistry, 4\(^{th}\) Ed., Longman, 1981;1371p.
[5] Collin C H. Microbiological Methods, Butter Wrths, London, 1964; 92.
[6] Gravestock M B and Ryley J F, Annual Reports in Medicinal Chem., 1984; 19: 127-136.
[7] Dunstan WR and Dymond TS. J Chem Soc., 1891; 59: 410-433.
[8] Ajay Kumar K, Govindaraju M, Jayaraoopa P and Vasanth Kumar G. Int J Pharma Bio Sci., 2013; 3(1); 91-101.
[9] Ajay Kumar K, Romuku N and Vasanth Kumar G. Int J Pharm Tech Res., 2013; 5(1): 239-248.
[10] Ajay Kumar K. Paviithra DM, Paviithra G and Vasanth Kumar G. Res J Pharm Tech., 2012; 5(12); 1490-1496.
[11] Jawalekar AM, Reubsaeit E, Rutjes Floris PIT and van Delft FL. Chem Commun., 2011; 47: 3198-3200.
[12] Sandeep B, Santosh K, Upadhye VP, Venkata PP and Debnath B. Tetrahedron Lett., 2009; 50; 3948-3951.

Shrivankumar K, Ravinder V, Chandra Sekhar V. Org Biomol Chem, 2011; 9: 7869-7876.
[13] Nagatosh N, Kazuya K, Shotaro H, Jun S, Kazuhiko S, Yumiko I, Malo N and Masahiro A, Org Biomol Chem., 2012; 10:1987-1991.
[16] Bhaskar Chakraborty, Manjit Singh Chhetri, Saurav Kafley and Amalesh Samanta. Indian J Chemistry., 2010; 49B: 209-215.

[17] Stokes BJ, Vogel CV, Urnezis LK, Pan M and Driver TG. Org Lett., 2010; 12(12): 2884-2887.

[18] Waldo JP and Larock RC. Org Lett., 2005; 7: 5203-5205.

[19] Ajay Kumar K, Lokanatha Rai KM and Umesh K. Journal Chem Res (S)., 2001; 436-438.

[20] Hemant S Chandak. Der Pharma Chem., 2012; 4(3): 1054-1057.

[21] Ajay Kumar K, Lokanatha Rai KM, Umesh KB and Prasad KR. Ind J Chem., 2001; 40B: 269-273.