WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 4, 814-821 Research Article ISSN 2278 – 4357

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF NOVEL ISOXAZOLINE

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Article Received on
24 Jan. 2018,
Revised on 14 Feb. 2018,
Accepted on 07 March 2018
DOI: 10.20959/wjpps20184-11201

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ABSTRACT

Chalcones were synthesized by the condensation product of acetophenone in combination with aromatic aldehydes in presence of strong base. It was found that the synthesized chalcones were having prominent role in modern coordination chemistry. The chalcone synthesized by base catalyzed condensation of 3-acetyl-6-methyl-2H-pyran-2,4-(3H) dione (DHA) with different aromatic aldehyde. These chalcones were used for synthesis of derivatives i.e. isoxazoline. The synthesized compounds were characterized by IR, ^{1}HNMR and mass spectral analysis. The derivatives were further used for the estimation of its biological properties. It was found that the derivative possesses efficient antimicrobial properties. From the study it was found that the synthesized compounds are efficient for further research work.

KEYWORDS: Dehydroacetic acid (DHA), Chalcone, 3-cinnamoyl-4-hydroxy-6-methyl-2-pyrones, IR, ^{1}HNMR, Antibacterial activity, Antifungal activity, Isoxazoline.

INTRODUCTION

Chalcones are the special ligand molecules that used for the synthesis of complexes with desired properties. The complexes are having variations in physical, chemical and biological properties. The existence of the α, β-unsaturated ketone moiety in chalcones is a common part found in a large number of biological active compounds[1], Therefore, chalcone derivatives from nature or synthetic origin exhibit diverse pharmacological activities, such as antimicrobial[2], antitumor[3], anticancer[4], radical scavenger[5] and inhibitor of topoisomerase I.[6]

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 industry. Numerous compounds containing isoxazole, pyrazole and pyrimidine moiety have been reported as active hypoglycemic[7], antidiabetic[8], antipyretics[9,11], analgesics[12,13], antiinflammatory, antiviral, antiallergic, anticancer agent, antidrepressant and antimicrobial agents which include antibacterial sulfonamides, semisynthetic penicillins and cephalosporines.[14,18] These discoveries led the scientist to attempt the synthesis of isoxazole, 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 activity.[19]

Isoxazolines were reported for their various biological activities. The reactive intermediate chalcones involved in their synthesis also exhibit wide range of biological activities. These properties were used in the research paper to find some novel compounds for pharmaceutical and agricultural industry.

RESULT AND DISCUSSION

The chalcones of DHA were synthesized by claisen-schmith condensation and characterized as good to excellent yield (scheme I) as shown in Table 1. The structures of all the compounds were established from IR, ^{1}HNMR and mass spectral analysis is mentioned above. The IR spectrum of chalcones gives a broad band for OH group at (3000-3125 cm^{-1}) sharp and strong bands were observed at 1700-1750 cm^{-1} for lactone carbonyl group. Another sharp band was observed at 1598-1650 cm^{-1} due to the presence of carbonyl group and carbon-carbon band of α, β unsaturated chalcone system.

The structures of synthesized compounds (MBOI to MBOV) were confirmed on the basis of spectral analysis. The IR spectrum of compound shows a broad band of 3420-3298 cm^{-1} OH group 2912-2965 cm^{-1} indicate the CH Str. in CH_2 / CH_3 group and 1720-1726 cm^{-1} for lactone carbonyl group sharp and strong band are observed. Another sharp band 1598-1622 cm^{-1} due to the presence of C=N in isoxazoline ring and 836-846 cm^{-1} due to the presence of NO in isoxazoline ring. Further in their ^{1}HNMR ($CHCl_3$) spectra the appearance of a signal at δ 3.2 -3.4 (1H, dd, H_A isoxazoline ring), δ 3.5–3.8 (1H, dd, H_B isoxazoline ring) δ 5.2-5.8 (1H, t, Hx isoxazoline ring) δ 14.2-14.8 (1H, S, OH) confirmed the presence of the isoxazoline ring. The physical characterization of derivatives is mentioned in Table 2.

Antimicrobial activity

The antimicrobial activity was tested against the bacterial and fungal species, the effective zone of inhibition were observed at each concentration as mentioned in Table 3. The results were compared with standard penicillin and the control was taken as DMSO. There was no antimicrobial activity of DMSO on microbial growth. Both the organism's i.e. bacteria and fungi showed maximum zone of inhibition that were 20 mm and 21 mm, respectively as shown in Table 3.

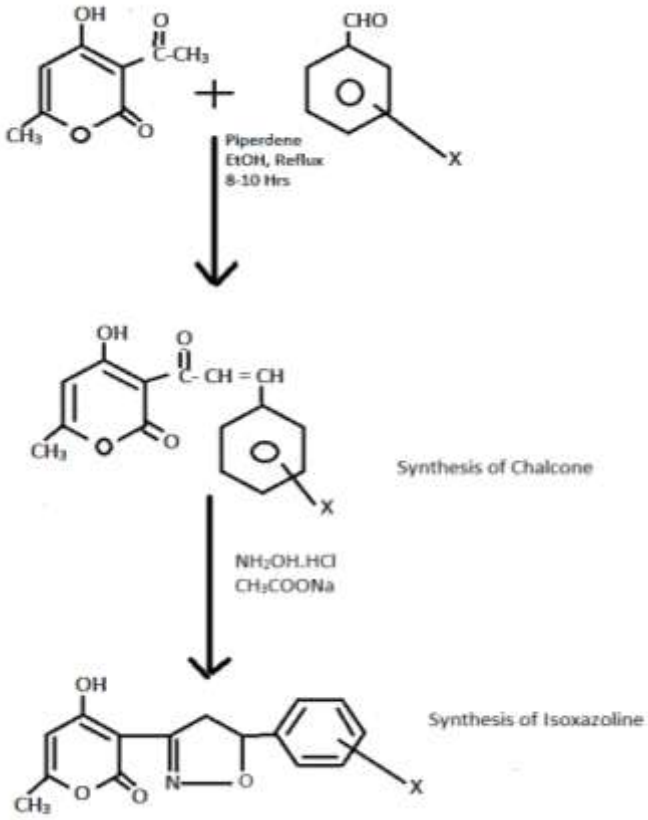


Fig. 1: Schematic representation of synthesized chalcone and Isoxazoline.

MATERIAL AND METHOD

Synthesis of substituted 3-Cinnamoyl-4-Hydroxy-6- Methyl-2-Pyrones (MBCI-V)

10 mmol solutions of dehydroacetic acid and the10 mmol of aromatic aldehyde were taken and in to that 8-10 drop of piperedine was added as a catalyst. The solutions was dissolved in 30 ml of ethanol solvent, the reaction mixture was then refluxed for a reaction time of 12-15 hrs. After reaction the compounds were checked by TLC. Then the mixture were filtered, dried and recrystallized with suitable solvent i.e. chloroform.[20]

The characterizations were carried out further of synthesized compounds. Melting points were determined in open capillary and are uncorrected. IR spectra were recorded on perkin Elmer R-X-IFT-IR spectrometer using potassium bromide pellet as standard, ^{1}HNMR's were determined on a New Avenue-500 MHz spectrometer against TMS as internal standard. The mass analysis was also carried out using Shimazu Machine. Purity of compounds was checked by thin layer chromatography (TLC).

General procedure for synthesis of isoxazoline

A mixture of chalcones (0.001 mol), hydroxyl amine hydrochloride (0.015 mol) and sodium acetate (0.002 mol) were dissolved in 15 ml ethanol, which was refluxed for 6 to 7 hrs. After completion of the reaction (checked by TLC). The compounds were synthesized by following procedure mentioned in Fig 1. The reaction mixture was cooled and poured on ice cold water. The separated solid product was filtered washed with cold water dried and recrystallized from ethyl alcohol. These synthesized derivatives were used for further analysis.[21]

Spectroscopic data of synthesized Isoxazoline derivatives (MBOI-MBOV)

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

IR (KBr, cm^{-1}); 3412 (OH str.), 2952 (C-H str. Of –CH_3), 1723 (C=O Lactone), 1615 (C=N str. of Isoxazoline ring), 1259 (C-N str. of Isoxazoline ring), 838 (N-O Str.).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.3 (3H, s, CH_3), 3.4 ($1H_A$, dd, -CH_2 Isoxazoline ring), 3.5 ($1H_B$, dd, -CH_2 Isoxazoline ring), 5.23 ($1H_X$, t, -CH Isoxazoline ring), 5.9 (1H, C^5 DHA), 6.2-7.8 (4H, m, Ar-H), 14.2 (1H, s, OH).

Mass (m/z): (M+1) 315.

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

IR (KBr, cm^{-1}): 3405 (OH str.), 2912 (C-H str. of CH_3), 1722 (C=O lactone), 1618 (C=N str. of Isoxazoline ring), 1270 (C-N str. of Isoxazoline ring), 840 (N-O Str.).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.3 (3H, s, CH_3), 3.9-4.0 (9H, s, 3xOCH_3), 3.3 ($1H_A$, dd, -CH_2 Isoxazoline ring), 3.5 ($1H_B$, dd, -CH_2 Isoxazoline ring), 5.7 ($1H_X$, t, -CH pyraz), 6.0 (1H, s, C^5 DHA), 6.4-7.9 (2H, m, Ar-H), 14.8 (1H, s, OH).

Mass (m/z): (M+1) 362.

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

IR (KBr, cm^{-1}): 3310 (OH Str.), 2965 (C-H str. of CH_3), 1720 (C=O lactone), 1610 (C=N str. of Isoxazoline ring), 1238 (C-N str. of Isoxazoline ring), 842 (N-O Str.).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.28 (3H, s, CH_3), 3.8 (3H, s, O-CH_3), 3.4 ($1H_A$, dd, CH_2 Isoxazoline ring), 3.58 ($1H_B$, dd, CH_2 Isoxazoline ring), 5.4 ($1H_X$, t, -CH Isoxazoline ring),6.1 (1H, s, C^5 DHA), 6.2-7.8 (4H, m, Ar-H), 14.8 (1H, s, OH).

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

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

IR (KBr, cm^{-1}): 3298 (OH str.), 2965 (C-H str. of CH_3), 1728 (C=O lactone), 1598 (C=N str. of Isoxazoline ring), 1242 (C-N str. of Isoxazoline ring), 846 (N-O Str.).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.2 (3H, s, CH_3), 4.0 (6H, s, 2xOCH3), 3.3 ($1H_A$, dd, CH_2 Isoxazoline ring), 3.8 ($1H_B$, dd, CH_2 pyraz), 5.8 ($1H_X$, t, CH Isoxazoline ring), 6.0 (1H, s, C^5 DHA), 6.4-7.6 (3H, m, Ar-H), 14.8 (1H, s, OH).

Mass (m/z): (M+1) 318.

MBPV:3-(4-hydroxy-6-methyl-2.oxa-2H-pyran-3-yl)-5-(2-florophenyl)-4,5-dihydro-2-Isoxazoline

IR (KBr, cm^{-1}): 3420 (OH str.), 2942 (C-H str. of CH_3), 1726 (C=O lactone), 1622 (C=N str. of Isoxazoline ring), 1279 (C-N str. of Isoxazoline ring), 836 (N-O Str.).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.2 (3H, s, CH_3), 3.2 ($1H_A$, dd, CH_2 Isoxazoline ring), 3.6 ($1H_B$, dd, CH_2 Isoxazoline ring), 5.4 ($1H_X$, t, CH Isoxazoline ring), 6.0 (1H, s, C^5 DHA), 6.8-8.0 (4H, m, Ar-H), 14.4 (1H, s, OH).

Mass (m/z): (M+1) 290.

Biological Activity

Antibacterial activity

The synthesized compounds were tested in *in vitro* for antimicrobial activity against bacterial isolates like *S. aureus*, *E. coli* and *Salmonella Typhi* and fungi species like *Fusarium oxysporum*, *Candida albicans* and *Aspergillus flavus.* The concentrations of compounds were taken as 150 µg/ml each. The antimicrobial activity was checked by agar plate diffusion method.[22,23] The concentrations used for activity was confirmed after estimating the MICs of each compound. The solvent used for assay was dimethyl sulfoxide (DMSO) which further diluted with water. Nutrient agar and PDA (Potato Dextrose Agar) was used as the growth medium for the bacterial and fungal species respectively. DMSO was used as a negative control. The results were compared with standard drug penicillin for antimicrobial activity by measuring the zone of inhibition in mm using 150 µg/mL. Antimicrobial activity was measured as a diameter of zone of inhibition (mm).[24]

Table 1: Percentage yield and melting point of substituted 3-Cinnamoyl-4-Hydroxy-6-Methyl-2-Pyrones.

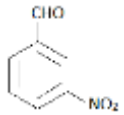
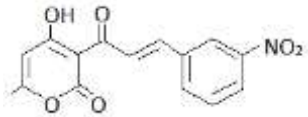
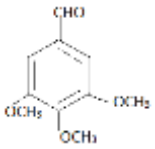
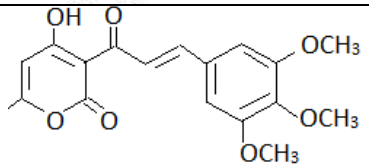
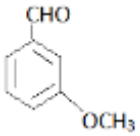
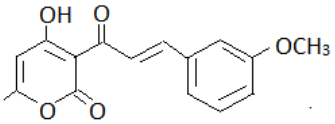
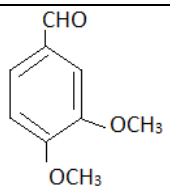
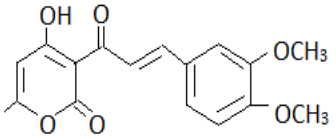
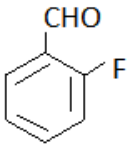
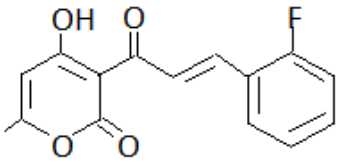
Entry	X	Product		Yield %	Melting point ºC
1		MBCI		70	190
2		MBCII		80	198
3		MBCIII		85	195
4		MBCIV		80	176
5		MBCV		84	160

Table 2: Physical data of isoxazoline derivation (MBOI-MOV).

Compounds	R'	Molecular Formula	M. P (ºC)	Yield %
MBO I	3-NO2	$C_{15}H_{10}O_6N_2$	220	65
MBO II	3,4,5-tri-OCH_3	$C_{18}H_{19}O_7N$	260	78
MBO III	3-OCH_3	$C_{16}H_{15}O_6N$	222	72
MBO IV	3,4-di-OCH_3	$C_{16}H_{15}O_6N$	180	74
MBO V	2-F	$C_{15}H_{12}O_4NF$	265	78

Table 3: Antimicrobial activity of Isoxazoline.

Compound	Bacteria (Zone of Inhibition in mm)			Fungi (Zone of Inhibition in mm)		
	A	**B**	**C**	**D**	**E**	**F**
MBO I	13	16	14	14	19	**21**
MBO II	15	17	12	15	17	19
MBO III	18	15	18	18	15	18
MBO IV	12	18	17	19	18	18
MBO V	14	**20**	19	14	20	16
Penicillin*	10	12	13	11	10	12

***standard,* A-** *S. aureus*, **B-** *E. coli*, **C-** *S. Typhi*, **D-** *Fusarium oxysporum*, **E-** *Candida albicans*, **F-** *Aspergillus flavus.*

CONCLUSION

In conclusion, we have reported that the synthesized chalcones derivatives using DHA (3-acetyl-6-methyl-2H-pyran-2,4-(3H) dione possessing a good to moderate biological properties. These compounds will be having application in pharmaceutical, agriculture, medical field for drug development.

ACKNOWLEDGEMENT

The authors are thankful to the director of IICT, Hyderabad and Dr. Makarand Kulkarni, Solapur University, Solapur for providing spectral analysis facilities to carry out research programme.

REFERENCES

1. Haripara K, Patel S, Joshi A and Paresh H, *Indian J Heterocycl Chem.*, 2004; 13: 221.
2. Wagner E, Becam L and Nowakowska E, *Bioorg Med Chem.*, 2004; 12: 265-272.
3. Ngaini Z, Siti M Haris-Fadzillah, Hasnain Hussain and Kamarulzamon Kamoruddin, *World J Chem.*, 2009; 4(1): 09-14.
4. Vogel A I, Textbook of Practical Organic Chemistry, 4th 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, Jayaroopa P and Vasanth Kumar G. Int J Pharma Chem Bio Sci., 2013; 3(1): 91-101.
9. Ajay Kumar K, Renuka N and Vasanth Kumar G. Int J Pharm Tech Res., 2013; 5(1): 239-248.
10. Ajay Kumar K, Lokeshwari DM, Pavithra G and Vasanth Kumar G. Res J Pharm Tech., 2012; 5(12): 1490-1496.
11. Jawalekar AM, Reubsaet E, Rutjes Floris PJT and van Delft FL. Chem Commun., 2011; 47: 3198-3200.
12. Sandeep B, Santosh K, Uppuleti VP, Venkata PP and Debnath B. Tetrahedron Lett., 2009; 50: 3948-3951.
13. Shravankumar K, Ravinder V, Chandra Sekhar V. Org Biomol Chem., 2011; 9: 7869-7876.
14. Nagatoshi N, Kazuya K, Shotaro H, Jun S, Kazuhiko S, Yumiko I, Maho N and Masahiro A. Org Biomol Chem., 2012; 10: 1987-1991.
15. Bhaskar Chakraborty, Manjit Singh Chhetri, Saurav Kafley and Amalesh Samanta. Indian J Chemistry., 2010; 49B: 209-215.
16. Stokes BJ, Vogel CV, Urnezis LK, Pan M and Driver TG. Org Lett., 2010; 12(12): 2884-2887.
17. Waldo JP and Larock RC. Org Lett., 2005; 7: 5203-5205.
18. Ajay Kumar K, Lokanatha Rai KM and Umesha K. Journal Chem Res (S)., 2001; 436-438.
19. Hemant S Chandak. Der Pharma Chem., 2012; 4(3): 1054-1057.
20. Ajay Kumar K, Lokanatha Rai KM, Umesha KB and Prasad KR. Ind J Chem., 2001; 40B: 269-273.
21. Maryam M and Gholam HM. E-Journal of Chemistry., 2012; 9(1): 425-429.
22. Vasanth Kumar G, Jayaroopa P, Bi Bi Ahmadi Khatoon, Mylarappa BN and Ajay Kumar K. Der Pharma Chem., 2012; 4(6): 2283-2287.
23. Scott ED and Jeffrey MK. J Org Chem., 2005; 70: 2839-2842.
24. Kadnor VA, Pandhare GR, Gadhave AG, Uphade BK. Rasayan J Chem., 2011; 4(2): 437-441.