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Synthesis and estimations of antimicrobial properties of novel pyrazoline derivatives

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Abstract
Chalcones are the condensation product of acetophenone in combination with aromatic aldehydes in the presence of strong base. Chalcones and their metal complexes having prominent role in modern coordination chemistry. These compounds possessing novel structural features, interesting spectral and magnetic properties, have been the subject of intensive research due to their importance in medical, agriculture, analytical, biological and industrial fields. A series of chalcones were prepared by claisen Schmidt condensation of substituted 3-cinnamoyl-4-hydroxy-6- methyl-2-pyrones were synthesized by base catalyzed condensation of 3-acetyl-4-hydroxy-6-methyl-2-oxa-2h-pyran (DHA) with different aromatic aldehyde. Biologically active pyrazoline derivatives were synthesized using ethanol via cyclization reaction with phenyl hydrazine hydrates from chalcones. The entire synthesized compounds were confirmed by IR, 1HNMR and Mass spectral analysis. It was found that the synthesized compounds possess standard pick at desired functional groups, protons and mass of compound. The newly synthesized pyrazoline derivatives (MBPI-MBPV) were tested for their antimicrobial activities which reflects

moderate to good activity against different strain of bacterial and fungi species. The pyrazoline derivatives synthesized in this research having applicability in the medical, pharmaceutical and agricultural filed.

Keywords: Chalcones, Phenyl Hydrazine Hydrate, Pyrazoline, Antimicrobial activities, IR, H'NMR and Mass spectra.

Introduction

Chalcones represent an important class of natural as well as synthetic products and some of them are having wide range of pharmaceutical activity such as antibacterial[1,2], antitumour[1,21], anticancer[1,3,5], antitubercular[2], antiinflammatory[1], antioxidant[1], antimalarial[1], antileishmanial[1] etc. The presence of α, β- unsaturated ketogroup in the chalcones is found to be amenable for their biological activity[4].

From the previous studies it was found that N containing heterocyclic compounds from chalcones possesses wide variety of activities such as potential activities like chalcone with efficiency[6]. Pyrazolines are the five member heterocyclic compounds containing two nitrogen atoms[7]. As all heterocycles are well known for their biological utilizations, pyrazolines also shows variety of biological applications[8] same as of chalcones. Taking in to consideration the broad spectrum of utilities one cannot ignore the existence of pyrazolines in the field of synthesis[9].

Therefore in the present work, chalcones have been prepared by condensing various ketones with aromatic aldehyde[10], the synthesis of some novel pyrazolines was also carried out for some beneficial use. Led by these retainers, it appeared the interest in synthesis of novel pyrazoline derivatives and screened for their antimicrobial activities.

Material and methods

The chalcones have been prepared according to the standard protocol of claisen-schimidt[11] condensation by condensing various ketones with aromatic aldehyde. The synthesis of novel pyrazolines using chalcones under basic condition in presence of ethanol as a solvent media[12]. The reaction is carried out in refluxing condition.

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

A solution of (10 mmol) of dehydroacetic acid of the aromatic aldehyde of (10mmol) and 8-10 drop of piperedine as a catalyst was dissolved in 30 ml of ethanol solvent, the

reaction mixture was refluxed for a reaction time 12-15 hrs. After reaction time check the TLC then filter the reaction mixture dried and recrystallized with suitable solvent as shown in (Fig1 Scheme I).

IR spectra were recorded in KBr spectrometer. ^{1}HNMR spectra were developed on Advance 300 MHz spectrometer with chloroform as a solvent and TMS internal standards chemical shift, the chemical shift values are expressed in part per million (ppm) downfield from the internal standards and signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplet) purity of the compound is checked by TLC plate using n-hexane and ethyl acetate as an eluent in the ratio of (7:3 v/v).

Spectroscopic data of synthesized chalcone (MBCI-MBCV)

MBCI: 1-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-3-(3-Nitrophenyl)-2-Propenone

IR (cm^{-1}, KBr): 3114 (OH), 3062 (CH aromatic), 2900 (CH_3), 1729 (Lactone C=O), 1648 (C=O), 1614 (CH=CH).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.30 (3H, s, CH_3), 6.0 (1H, s, C^5 DHA), 7.3 (1H, dd, -C=OCH), 8.2 (1H, dd, =CH-Ar), 6.5-8.4 (4H, m, Ar-H), 14.4 (1H, s, OH).

MBCII: 1-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-3-(3-3,4, 5-Trimethoxy)-2-Propenone

IR (cm^{-1}, KBr): 3121 (OH), 2954 (CH_3), 1726 (Lactone C=O), 1651 (C=O), 1598 (CH=CH)

^{1}HNMR ($CDCl_3$, δ/ ppm), 2.4 (3H, s, CH_3), 3.93 (9H, s, 3xOCH_3), 6.0 (1H, s, C^5 DHA), 7.9 (1H, dd, -C=OCH), 8.2 (1H, dd, =CH-Ar) 6.6-7.2 (2H, m, Ar-H), 13.2 (1H, s, OH).

MBCIII: 1-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-3-(3-methoxyphenyl)-2, Propenone

IR (cm^{-1}, KBr): 3117 (OH), 2969 (CH_3), 1722 (Lactone C=O), 1655 (C=O), 1597 (CH=CH), 1512 (CH=CH-Ar), 1487 (CH=CH).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.1 (3H, s, CH_3); 3.9 (3H, s, OCH_3), 6.0 (1H, s, C^5 DHA), 7.9 (1H, dd, C=OCH), 8.2, (1H, dd, =CH-Ar), 6.2 -7.4 (4H, m, Ar-H), 14.6 (1H, s, OH).

MBCIV: 1-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-3-(3,4-Dimethoxyphenyl)-2-Propenone

IR (cm^{-1}, KBr): 3104 (OH), 2981 (CH_3), 1715 (Lactone C=O), 1658 (C=O), 1589 (CH=CH).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.4 (3H, s, CH_3), 3.9-4.0 (6H, s, 2xOCH3), 6.0 (1H, s, C^5 DHA), 7.9 (1H, dd, C=OCH), 8.3 (1H, dd, =CH-Ar), 6.8-7.3 (3H, m, Ar-H), 16.4 (1H, s, OH).

MBCV: 1-(4-hydroxy-6-methyl-2-oxa-2H-Pyran-3-yl)-3-(2-Fluorophenyl)- 2-Propenone

IR (cm^{-1}, KBr): 3103 (OH), 2973 (CH_3), 1719 (Lactone C=O), 1646 (C=O), 1608 (CH=CH),

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.3 (3H, s, CH_3), 6.0 (1H, s, C^5 DHA), 7.8 (1H, dd, C=OCH), 8.4 (1H, dd, =CH-Ar), 7.1-8.2 (4H, m, Ar-H), 15.8 (1H, s, OH).

General procedure for synthesis of phenyl pyrazoline

A mixture of chalcone (0.001 mol) and phenyl hydrazine hydrated (0.002 mol) in 10 ml ethanol was refluxed for 3 hours. After completing of reaction (monitored by TLC), the reaction mixture was distilled off to remove the excess solvent and then it was poured into crushed ice[13] as shown in (Fig1 Scheme II). The solid obtained was washed with water and recrystallized with the help of ethanol. The compounds were stored at room temperature in vials.

Spectroscopic data of synthesized pyrazoline derivatives (MBPI-MBPV)

MBPI: 3-(4-hydroxy-6-methyl-2oxa-2H pyran -3-yl) - 5- (3-Nitroptenyl) -1-phenyl - 4, 5- dihydro-2-Pyrazoline

IR (KBr, cm^{-1}); 3327 (OH str.), 2970 (C-H str. Of –CH_3), 1722 (C=O Lactone), 1612 (C=N str. of pyrazoline ring), 1575, 1532, 1447 (C=C str. of aromatic ring), 1230 (C-N str. of pyrazoline ring), 751 (C-H bending)

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.2 (3H, s, CH_3), 3.1 (1H, dd, -CH_2 pyraz), 3.9 (1H, dd, -CH_2 pyraz), 5.2 (1H, t, -CH pyraz), 6.0 (1H, C^5 DHA), 6.1-8.2 (9H, m, Ar-H), 13.4 (1H, s, OH)

Mass (m/z): (M+1) 392

MBPII:3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(3,4,5-trimethoxyphenyl)-1-phenyl-4,5-dihydro-2-pyrazoline

IR (KBr, cm^{-1}): 3376 (OH str.), 2980 (C-H str. of CH_3), 1720 (C=O lactone), 1614 (C=N str. of pyrazoline ring), 1577, 1531, 1497 (C=C str. of aromatic ring), 1232 (C-N str. of pyrazoline ring), 750 (OH bending).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.2 (3H, s, CH_3), 3.8-3.9 (9H, s, 3xOCH_3), 3.2 (1H, dd, -CH_2 pyraz), 3.6 (1H, dd, -CH_2 pyrazoline), 5.6 (1H, t, -CH pyraz), 5.9 (1H, s, C^5 DHA), 6.0-7.9 (7H, m, Ar-H), 13.5 (1H, s, OH)

Mass (m/z): (M+1) 437

MBPIII: 3-(4-hydroxy-6-methyl-2-oxa-2H-pyran-3-yl)-5-(3-Methoxyphenyl)--1-phenyl-4, 5-dihydro-2- pyrazoline

IR (KBr, cm^{-1}): 3330 (OH Str.), 2970 (C-H str. of CH_3), 1730 (C=O lactone), 1610 (C=N str. of pyrazoline ring), 1585, 1530, 1448 (C=C str. of aromatic ring), 1228 (C-N str. of pyrazoline ring), 749 (C- H bending).

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.3 (3H, s, CH_3), 3.3 (1H, dd, CH_2 pyraz), 3.8 (1H, dd, CH_2 pyraz), 5.5 (1H, t, -CH pyraz),6.0 (1H, s, C^5 DHA), 6.2-8.0 (9H, m, Ar-H), 14.4 (1H, s, OH)

Mass (m/z): (M+1) 374

MBPIV:3-(-hydroxy-6-methyl-2-oxa-2H-pyran-3-Yl)-5-(3,4,-dimethoxy phenyl)-1-phenyl-4, 5-dihydro-2-pyrazoline

IR (KBr, cm^{-1}): 3391 (OH str.), 2975(C-H str. of CH_3), 1725 (C=O lactone), 1608 (C=N str. of pyrazoline ring), 1595, 1528, 1485 (C=C str. of aromatic ring), 1230 (C-N str. of pyrazoline ring), 752 (C-H bending)

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.5 (3H, s, CH_3), 3.8-3.9 (6H, s, 2xOCH3), 3.4 (1H, dd, CH_2 pyraz), 3.5 (1H, dd, CH_2 pyraz), 5.7 (1H, t, CH pyraz), 6.0 (1H, s, C^5 DHA), 6.2-8.2 (8H, m, Ar-H), 14.2 (1H, s, OH)

Mass (m/z): (M+1) 407

MBPV:3-C4-hydroxy-6-methyl-2.oxa-2H-pyran-3-yl)-5-(2-florophenyl)1-phenyl-4, 5-dihydro-2-pyrazoline.

IR (KBr, cm^{-1}): 3405 (OH str.), 2985 (C-H str. of CH_3), 1730 (C=O lactone), 1596 (C=N str. of pyrazoline ring), 1575, 1520, 491 (C=C str. of aromatics ring), 1228 (C-N str. of pyrazoline ring), 748 (CH bending)

^{1}HNMR ($CDCl_3$, δ/ ppm): 2.3 (3H, s, CH_3), 3.6 (1H, dd, CH_2, puraz), 3.9 (1H, dd, CH_2 pyraz), 5.8 (1H, t, CH pyraz), 6.0 (H, s, C^5 DHA), 6.4-8.4 (9H, m, Ar-H), 14.4 (1H, s, OH)

Mass (m/z): (M+1) 365

Biological Activity

Antibacterial activity

The synthesized compounds were tested in *in vitro* for antimicrobial activity against bacterial species like *S. aureus*, *E. coli*, *S. Typhi* and fungi species like *Fusarium oxysporum* , *Candida albicans* and *Aspergillus flavus* at the concentrations 100μg/mL each, by agar plate diffusion method. The concentrations used for screening were 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 control. The results were compared with

standard drug penicillin for antimicrobial activity by measuring the zone of inhibition in mm at 100 µg/mL. Antimicrobial activity was measured as a function of diameter of zone of inhibition (mm)[14].

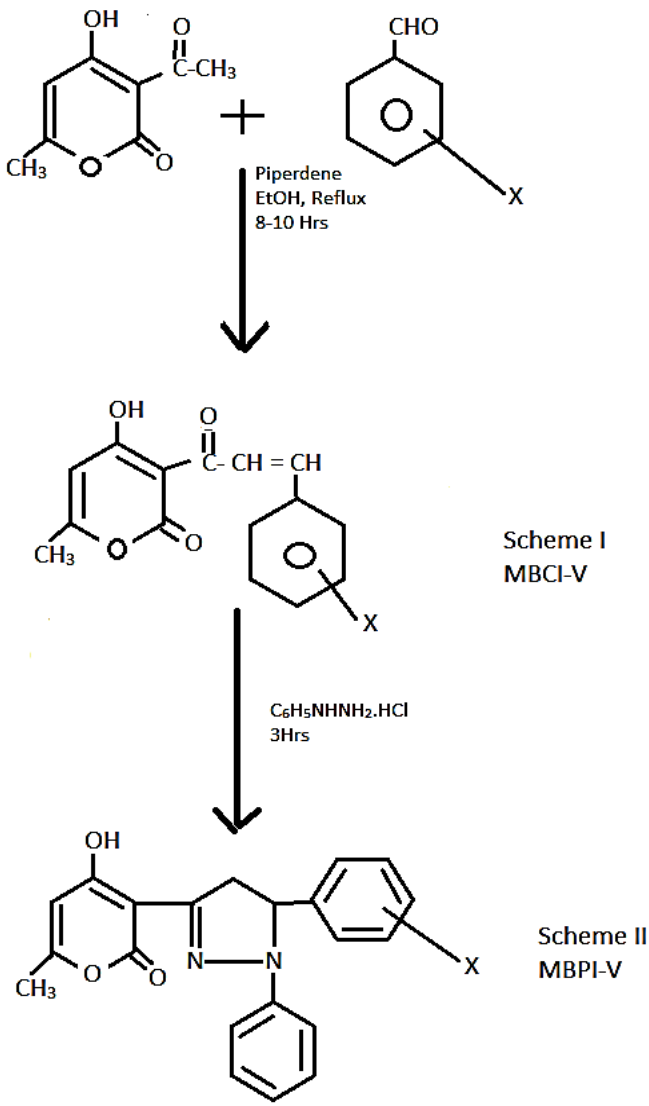

Fig. 1 Schematic representation of synthesized schemes I and II

Results and Discussions

The chalcones of DHA were synthesized by claisen schmith condensation in good to excellent yield (scheme I) as shown in Table 1. The structures of all the compounds were established from IR and ^{1}HNMR spectral analysis is mentioned above. The IR spectrum of compound MBCI to MBCV shows 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 observed at 1598-1650 cm^{-1} due to the presence of carbonyl group

and carbon-carbon band of α, β unsaturated chalcone system. The ^{1}HNMR spectra of MBCI to MBCV showed a characteristic singlet due to C^{5}-H proton δ 5.9-6.0 ppm for lactone unit. We also noted that the olefinic protons of reactive α, β unsaturated keto function occur as doublet around 7.9-8.4 respectively and broad singlet around at δ13.5-16.5 OH group of Lactone unit[15].

A series of some novel phenyl pyrazoline derivatives were synthesized by refluxing chalcone for the synthesis of phenyl pyrazoline have been investigated[16]. The presence of nitro methoxy, bromo, fluoro, chloro and methyl group in different position of benzene ring of the chalcones and the use of phenyl hydrazine hydrate resulted in synthesis of new phenyl pyrazoline derivatives with significantly high yield as mentioned in Table 2.

The structure of synthesized compound (MBP I-V) were confirmed on the basis of spectral analysis The IR spectrum of (MBP I–V) exhibited a band due to 2970-2980 (CH_3), 1720-1720 (lactone C=O), 1612-1614 (C=N), 3327-3376 (-OH), 1422 (CH_2 of pyrazoline ring). Further in their ^{1}HNMR ($CHCl_3$) spectra the appearance of a signal at δ 3.1-3.2 (1H, dd, H_A pyrazoline), δ 3.6-3.9 (1H, dd, H_B, pyrazoline), δ 5.2-5.6 (1H, t, H_X pyrazoline) δ 13.4-13.5 (1H , s, -OH) confirms the presence of the phenyl pyrazoline ring[17].

All the synthesized compounds screened for antibacterial activity and it was found that there is significant effect on the inhibition zone of microbial growth. The control showed no activity against the strains of microorganisms used. Both the organism's i.e bacteria and fungi showed maximum zone of inhibition that were 16 mm and 17 mm, respectively. Other strains were also tested against the standard drug[18, 19,20]. The results are given in Table 3.

Table 1. Physical properties of chalcones

Compound	R'	Molecular formula	M. P (ºC)	Yield %
MBCI	3-NO_2	$C_{15}H_{11}O_6N$	190	70
MBCII	3,4,5-OCH_3	$C_{18}H_{18}O_7$	198	80
MBCIII	3- OCH_3	$C_{16}H_{14}O_5$	195	82
MBCIV	3, 4-OCH_3	$C_{17}H_{16}O_6$	176	80
MBCV	2-F	$C_{15}H_{11}O_4F$	160	84

Table 2. Physical properties of pyrazoline derivatives (MBPI-V)

Compound	R'	Molecular formula	M. P (ºC)	Yield %
MBPI	3-NO_2	$C_{21}H_{17}O_5N_3$	215	52
MBPII	3,4,5-OCH_3	$C_{24}H_{24}O_6N_2$	235	58

MBPIII	3- OCH_3	$C_{22}H_{17}O_4N_2$	211	57
MBPIV	3, 4-OCH_3	$C_{23}H_{22}O_5N_2$	200	56
MBPV	2-F	$C_{21}H_{17}O_3N_2F$	240	52

Table 3. Antimicrobial activity of pyrazoline derivatives (MBPI-MBPV)

Compound	**Bacteria** (Zone of Inhibition in mm)			**Fungi** (Zone of Inhibition in mm)		
	A	**B**	**C**	**D**	**E**	**F**
MBPI	15	16	12	16	10	13
MBPII	12	10	14	15	16	15
MBPIII	13	20	16	17	12	14
MBPIV	14	11	10	11	14	12
MBPV	11	10	15	18	11	11
Penicillin	11	9	9	9	12	9

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 some novel pyrazoline compound shows considerable antimicrobial activity against tested bacteria and fungi species comparatively with standard used in study. In this study the synthesized pyrazoline derivatives will be having importance in the pharmacophoric possession and it's may provide us the fruitful results in biological and medicinal purposes. So these compounds will be having importance in the medical and agriculture field for management of diseases and pest.

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