Synthesis, Characterization and Antimicrobial Activity of Novel Chalcone series and its Isoxazole Derivatives

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ABSTRACT

A new series of chalcones have been prepared by the Claisen-Schmidt condensation. A novel series of isoxazole derivatives have been synthesized by the reaction of respective chalcones with hydroxylamine hydrochloride. The compounds were characterized by elemental analysis and mass, where spectral study of compound **1f, 1g, 2a** and **2h** also carried out by FTIR, ^{1}H NMR, ^{13}C NMR, and MS techniques. All synthesized compounds assayed for their antibacterial activity against *S. Aureus MTCC-96, B. Subtilis MTCC-441, E. Coli MTCC-443, S. Typhi MTCC-98* and antifungal activity against *A. Niger MTCC-282* and *A. Clavatus MTCC-1323* at different concentrations and compared with standard drugs. The minimum inhibition concentration (MIC) of the compounds were studied by the micro broth dilution method. **1b, 1c, 1d, 1h and 2e** showed moderate to comparable antibacterial activity against *E. Coli, S.Typhi, B. Subtillis* and *S. Aureus*. All of these substituted chalcone compounds did not show antifungal activity but its isoxazole derivatives **2e** and **2h** showed comparable anti fungal activity.

Keywords: *Isoxazole, Chalcone, Pyrazole carbaldehyde, Microbial activity*

INTRODUCTION

Chalcones have attracted researchers since last many decades due to their broad spectrum of biological activity like antibacterial[1], acetylcholinesterase inhibitor[2], Antitubercular[3], Anticancer[4], antidiabetic, anti-infective, anti-inflammatory, anti-oxidant, antiaging[5]. Chalcones have applications as mediator in synthesis of various organic compounds[6]. The α, β unsaturated propenone linkage may responsible for their broad spectrum of biological activity and their applications in various organic synthesis. A literature survey shows that heteroaromatic ring containing chalcones exhibits excellent biological activities[7,8]. Pyrazolic chalcones were reported for their potential as antimicrobial and antioxidant agents. Pyrazol derivatives exhibits antibacterial, antifungal, herbicidal, insecticidal and many other biological activities[9]. Isoxazoline compounds shows various pharmacological activities like antibacterial, antibiotic, antitumour, antifungal, analgesic, antituberculosis and anti-inflammatory[10-12].

MATERIALS AND METHODS

Thin-layer chromatography was accomplished on 0.2-mm pre coated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm). IR spectra were recorded on a SHIMADZU-FTIR-8400 spectrophotometer using DRS probe over frequencies ranging from 4000-400 cm^{-1}. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a BRUKER AVANCE II spectrometer in DMSO-d_6 as solvent and TMS as an internal standard. ^{13}C (100 MHz) NMR were recorded on 100 MHz spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a SHIMADZU GCMS-QP 2010 mass spectrometer. Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected. The chemicals used in this work were purchased from Merck and Spectrochem Chemical Companies. All chemicals were reagent grade and used without further purification, and all solvents were freshly distilled before use.

EXPERIMENTAL SECTION

General process for the synthesis of Chalcones (1a-h)

A series of chalcone **(1a-h)** were prepared by using 100 mL RBF containing a mixture solution of 5-chloro-1-phenyl-3-propyl-1*H*-pyrazole-4-carbaldehyde **(1) (**1 mmol**)** and respective para substituted acetophenone **(2)** (1 mmol) in 30 mL methanol. Followed by addition of 10 mL 10% KOH solution. Then reaction mixture was stirred for 5-10 h and kept overnight at room temperature. Then reaction mixture was quenched on to ice cold water and acidified with aqueous 10% HCl solution. Crude product was extracted using Ethyl acetate (25 mL), and organic layer was washed with water and organic solvent was evaporated using rotary evaporator to obtain solid compound. The product was purified by recrystallization in methanol. The compound was characterized by various techniques as mentioned in Table **1**.

General process for the synthesis of 5-(5-chloro-1-phenyl-3-propyl-1H-pyrazol-4-yl)-3-aryl-4,5-dihydroisoxazole (2a-h).

The compounds substituted 4,5-dihydroisoxazole **(2a-h)** obtained in a 100 mL round bottomed flask containing (1 mmol) respective chalcone compound **(1a-h)** and (1.1 mmol) hydroxylamine hydrochloride **(3)** in 20 mL ethanol was placed in a water bath and 10 % ethanolic potassium hydroxide solution (2 mL) was added drop wise at room temperature with stirring. Then reaction mixture temperature was raised to reflux with stirring for 6-14 h. The progress of reaction was monitored by TLC. After completion of reaction, reaction mixture was quenched on to ice cold water. Crude product was extracted using Ethyl acetate (25 mL) and organic layer was washed with water and organic solvent was evaporated using

rotary evaporator to obtain solid compound. The product was purified by recrystallization in methanol. The compound was characterized by various techniques as mentioned in Table **1**.

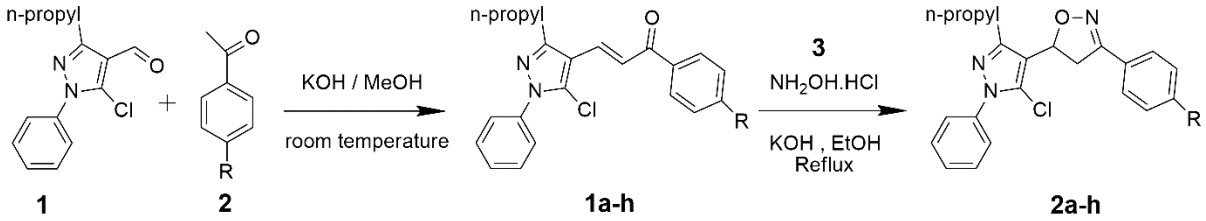

Figure 1: Synthesis of substituted chalcones **(1a-h)** and its 4,5-dihydroisoxazole derivatives **(2a-h)**

Table 1: Substrate scope

Code	R	M.F.	M.W.	M.P.	% yield	*R_f
1a	H	$C_{21}H_{19}ClN_2O$	350	168-170	87	0.69
1b	OH	$C_{21}H_{19}ClN_2O_2$	366	202-204	85	0.78
1c	OCH_3	$C_{22}H_{21}ClN_2O_2$	380	189-191	82	0.74
1d	F	$C_{21}H_{18}ClFN_2O$	368	161-163	71	0.79
1e	Cl	$C_{21}H_{18}Cl_2N_2O$	384	172-174	75	0.84
1f	Br	$C_{21}H_{18}BrClN_2O$	430	154-156	78	0.76
1g	NO_2	$C_{21}H_{18}ClN_3O_3$	395	181-183	88	0.64
1h	NH_2	$C_{21}H_{20}ClN_3O$	365	192-194	79	0.70
2a	H	$C_{21}H_{20}ClN_3O$	365	184-186	76	0.72
2b	OH	$C_{21}H_{20}ClN_3O_2$	381	166-168	68	0.79
2c	OCH_3	$C_{22}H_{22}ClN_3O_2$	395	196-198	71	0.76
2d	F	$C_{21}H_{19}ClFN_3O$	383	174-176	69	0.81
2e	Cl	$C_{21}H_{19}Cl_2N_3O$	399	185-186	64	0.85
2f	Br	$C_{21}H_{19}BrClN_3O$	445	136-139	70	0.78
2g	NO_2	$C_{21}H_{19}ClN_4O_3$	410	161-164	73	0.69
2h	NH_2	$C_{21}H_{21}ClN_4O$	380	149-151	57	0.74

*Hexane : Ethyl acetate (80:20)

Table 2: Antimicrobial activity of synthesised compounds:

Compound	Minimum inhibition concentration (μg/mL)					
	Gram-positive		Gram-negative		Fungal species	
	B. subtillis	***S. aureus***	***E. coli***	***S. typhi***	***A. niger***	***A.clavatus***
1a	250	500	500	1000	250	500
1b	200	250	100	200	500	500
1c	25	50	50	100	250	250
1d	200	100	100	200	500	250
1e	500	250	500	500	1000	500
1f	1000	1000	500	1000	>1000	1000
1g	>1000	1000	500	500	1000	1000
1h	100	100	100	250	500	1000
2a	1000	>1000	>1000	1000	500	500
2b	500	1000	250	500	250	200
2c	250	250	500	1000	250	500
2d	250	200	100	200	200	200
2e	100	200	200	100	100	100
2f	1000	500	250	500	250	500
2g	500	1000	500	>1000	500	500
2h	250	200	100	250	100	100
Ciprofloxacin	25	25	50	50	-	-
Norfloxacin	12.5	12.5	100	100	-	-
Nystatin	-	-	-	-	100	100
Griseofulvin	-	-	-	-	100	100

Biological Testing

A series of chalcone **(1a-h)** and it's 4,5-dihydroisoxazole **(2a-h)** derivatives were screened for their *invitro* antibacterial and antifungal activities following micro broth dilution method [13-15]. Antibacterial activity was screened against gram-negative (*Escherichia coli, Salmonella typhi*) and gram-positive (*Bacillus subtillis, Staphylococcus aureus*) microorganisms where antifungal activity was screened against *Aspergillus niger, and Aspergillus clavatus* microorganisms. The standard drugs used for this study were Ciprofloxacin and Norfloxacin for antibacterial screening, Nystatin and Griseofulvin for antifungal screening. Mueller Hinton Broth was used as a nutrient medium for bacteria and for fungal growth Sabouraud Dextrose Broth was used. By comparing the turbidity, inoculums size 108 CFU/mL was adjusted for test strain. The test performed in the form of primary and secondary screening. Each synthesized compounds under investigation and

standard drugs solution were diluted to obtain 2000 µg/mL concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/mL concentrations of the compounds were used by successive dilution. The compounds found to be active in this primary screening were further screened in secondary screening where 200, 100, 50, 25, 12.5 and 6.25 µg/mL concentrations were used. The inoculated wells were incubated at 37ºC for 24 h in a humid atmosphere The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards.

RESULTS AND DISCUSSION

Claisen-Schmidt condensation of 5-chloro-1-phenyl-3-propyl-1H-pyrazole-4-carbaldehyde **(1)** with substituted acetophenone **(2)** in polar solvent like methanol gave substituted chalcones **(1a-h)** by using KOH as base catalyst. These substituted chalcones **(1a-h)** were refluxed with hydroxylamine hydrochloride in presence of alkali in ethanol to afford the corresponding isoxazole derivatives **(2a-h)**. These compounds were characterized by elemental analysis and mass, where spectral study of compound **1f, 1g, 2a** and **2h** also carried out by FTIR, ^{1}H NMR, ^{13}C NMR, and MS techniques to elucidate the synthesized compounds structure.

Compound Characterizations

1-(4-bromophenyl)-3-(5-chloro-1-phenyl-3-propyl-1H-pyrazol-4-yl)prop-2-en-1-one (1f): Yield, 78%, m.p. 154-156ºC; IR (cm^{-1}): 3076 (C-H stretching of aromatic ring), 2969 (C-H stretching of aliphatic),1751 (C=O stretching), 1656 (C=C stretching of enone), 1590 (C=C stretching of aromatic ring), 1495 (C=C stretching of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.995-1.032 (t, 3H,n-propyl-C<u>H</u>$_3$, J = 7.2 Hz),1.693-1.785 (m, 2H, n-propyl-C<u>H</u>$_2$CH$_3$), 2.858-2.896 (t, 2H, n-propyl-C<u>H</u>$_2$CH$_2$, J = 7.6 Hz), 7.544-7.680 (m, 7H, aromatic <u>H</u>, C<u>H</u>=C<u>H</u>), 7.798-7.819, 7.982-8.004 (dd, 4H, aromatic <u>H</u>). ^{13}C NMR (DMSO-d$_6$) δ ppm: 13.743, 20.793, 29.160, 113.051 120.168, 125.073, 127.184, 128.345, 128.940, 129.341, 130.235, 131.964, 132.732, 136.568, 137.197, 153.444, 188.017; *m/z : 428; Anal. Calcd. For* $C_{21}H_{18}BrClN_2O$: C, 58.69; H, 4.22; N, 6.52; Found: C, 58.95; H, 4.25; N, 6.78.

3-(5-chloro-1-phenyl-3-propyl-1H-pyrazol-4-yl)-1-(4-nitrophenyl)prop-2-en-1-one (1g): Yield, 88%, m.p. 181-183ºC; IR (cm^{-1}): 3069 (C-H stretching of aromatic ring), 2970 (C-H stretching of aliphatic),1745 (C=O stretching), 1638 (C=C stretching of enone), 1576 (C=C stretching of aromatic ring), 1486 (C=C stretching of aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.999-1.035 (t, 3H, n-propyl-C<u>H</u>$_3$, J = 7.2 Hz),1.717-1.772 (m, 2H, n-propyl-C<u>H</u>$_2$CH$_3$), 2.870-2.907 (t, 2H, n-propyl-C<u>H</u>$_2$CH$_2$, J = 7.6 Hz), 7.551-7.713 (m, 7H, aromatic <u>H</u>, C<u>H</u>=C<u>H</u>), 8.260-8.281, 8.393-8.414 (dd, 4H, aromatic <u>H</u>). ^{13}C NMR (DMSO-d_6) δ ppm: 13.743, 20.769, 29.148, 112.981, 120.168, 123.972, 125.065, 128.666, 128.994, 129.356,

129.607, 133.697, 137.136, 142.429, 149.776, 153.599, 188.084; *m/z :* 395*; Anal. Calcd. For* $C_{21}H_{18}ClN_3O_3$: C, 63.72; H, 4.58; N, 10.62; Found: C, 63.58; H, 4.52; N, 10.67.

5-(5-chloro-1-phenyl-3-propyl-1H-pyrazol-4-yl)-3-phenyl-4,5-dihydroisoxazole (2a):

Yield 76%; m.p. 184-186^{0}C; IR (cm^{-1}): 3063 (C-H stretching of aromatic ring), 2962 (C-H stretching of aliphatic), 1612 (C=C stretching of aromatic ring), 1496 (C=C stretching of aromatic ring), 1303 (C-O stretching of heterocyclic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.936 (t, 3H, n-propyl-C<u>H</u>$_3$), 1.664 (m, 2H, n-propyl-C<u>H</u>$_2$CH$_3$), 5.655 (bs, 1H, heterocy. <u>H</u>), 7.261-7.716 (m, 10H, aromatic <u>H</u>); ^{13}C NMR (DMSO-d_6) δ ppm: 13.736, 21.737, 29.148, 74.359, 119.087, 119.844, 125.070, 126.430, 128.764, 128.863, 129.259, 129.815, 137.434, 138.957, 156.636; m/z = 362.9; *Anal. Calcd. For* $C_{21}H_{20}ClN_3O$: C, 68.94; H, 5.51; N, 11.49; Found: C, 68.85; H, 5.52; N, 11.56.

4-(5-(5-chloro-1-phenyl-3-propyl-1H-pyrazol-4-yl)-4,5-dihydroisoxazol-3-yl)aniline (2h):

Yield 57%; m.p. 149-151^{0}C; IR (cm^{-1}): 3348-3225 (N-H stretching of primary amine), 3063 (C-H stretching of aromatic ring), 2955 (C-H stretching of aliphatic), 1612 (C=C stretching of aromatic ring), 1504 (C=C stretching of aromatic ring), 1296 (C-O stretching of heterocyclic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.995 (t, 3H, n-propyl-C<u>H</u>$_3$), 1.715 (m, 2H, n-propyl-C<u>H</u>$_2$CH$_3$), 2.902 (t, 2H, n-propyl-C<u>H</u>$_2$CH$_2$), 5.341 (bs, 1H, heterocy. <u>H</u>), 6.556-6.705 (m, 2H, aromatic <u>H</u>), 6.979-7.162 (m, 2H, aromatic <u>H</u>), 7.465-7.650 (m, 4H, aromatic <u>H</u>), 8.023(1H, aromatic <u>H</u>); ^{13}C NMR (DMSO-d_6) δ ppm: 13.728, 21.078, 29.610, 99.177, 111.431, 114.369, 115.820, 124.982, 125.302, 125.921, 129.034, 129.244, 129.351, 129.548, 137.219, 148.937, 151.435, 162.542; m/z = 379.9; *Anal. Calcd. For* $C_{21}H_{21}ClN_4O$: C, 66.22; H, 5.56; N, 14.71; Found: C, 65.89; H, 5.51; N, 14.65.

Antimicrobial Activity

Antimicrobial activity performed by micro broth dilution method. The MIC value obtained for synthesized compounds **1a-h**, **2a-h** and for Ciprofloxacin, Norfloxacin, Nystatin and Griseofulvin as standard drug. In which compound **1b** showed moderate activity aginst *E. coli*. and *S. typhi*. Compound **1h** showed good activity against *B. subtillis* and *S. aureus*. Compound **1d** and **2e** found moderate active and compound **1c** found comparable active against all four bacterial strains *E. coli, S. typhi, S. aureus* and *B. subtillis*. Substituted chalcone compounds did not show anti fungal activity where its isoxazole derivatives **2e** and **2h** found comparable active against fungal species *A. niger and A.clavatus.*

CONCLUSION

Substituted chalcones and its isoxazole derivatives were synthesized and characterized by spectral techniques. They also screened for antibacterial activity and anti fungal activity against selected microbes and compared with standard drug Ciprofloxacin, Norfloxacin, Nystatin and Griseofulvin. In which some of the substituted chalcones and its isoxazole

derivatives found moderate to comparable active against selected bacterial strains where isoxazole derivatives **2e** and **2h** showed comparable anti fungal activity.

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