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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NEW DIHYDROPYRIMIDINONE'S CHALCONES.

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Key words

Bigineeli reaction Dihydropyrimidinone, Claiesn-Schmidt condensation, Chalcones, Antibacterial activity, Antifungal activity

ABSTRACT

Biginelli reaction is the classical route for the synthesis of dihydro pyrimidinones, which were possess wide range of pharmacological activities such as antimicrobial, antitumor, antitubercular, anti-inflammatory, antiviral, antimalarial and potent calcium channel modulators, antihypertensive activity. In this present work a series of new dihydropyrimidinones were synthesized by condensation of urea, acetylacetone with different aldehydes in presence citric acid at 80^{o}c (1a-1j). Chalcones are natural or synthetic compounds containing 1, 3 diaryl-prop-2-ene-1-one system, which is considered as an open chain flavonoidal structure. Pharmacologically chalcones were known to display wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic, antioxidant, and anti tubercular, anti tumor activities. Dihydropyrimidinones and chalcones both have therapeutic importance. By synthesizing the dihydropyrimidinone chalcone derivatives may produce potent bioactive compounds. Dihydropyrimidinone containing chalcones (2a-2e) were synthesized by Claisen-Schmidt condensation, aryl ketone (1j) condensed with different aldehydes in presence of alkali solution at room temperature with continuous stirring for 24 hours and synthesized compounds were screened for antibacterial and antifungal activities. All the synthesized compounds shown mild to moderate antimicrobial activity.

INTRODUCTION

Pyrimidinones are remarkable scaffolds having wide range of biological importance. Thiamine, Uracil and Cytocine are pyrimidine bases and they are found in DNA and RNA respectively, which plays vital role in biological cell function. In recent years dihydropyrimidinones received more attention because they exhibit wide range of biological and therapeutic activities such as anti-tumor, anti-viral, antimicrobial, anti-inflammatory activities[1], potent calcium channel modulators, anti hypertensive agents and neuropeptide Y (NPY) antagonists[2]. Dihydropyrimidinones also found in many marine natural products including Betzelladine is an alkaloid which process HIV_{gp}-120-CD_4 inhibiting activity[3].

Chalcones are natural or synthetic compounds containing 1, 3 diaryl-prop-2-ene-1-one system, which is considered as an open chain flavonoidal structure. Chalcones are the precursors for synthesis

of many flavoinoidal compounds which have great therapeutical value. Pharmacologically chalcones were known to display wide range of biological activities such as antimicrobial[4], anti-inflammatory[5], antiplasmodial[6], immunosuppresion[7], antioxidant[8], antihyperglycemic activity[9] and anti-tumor [10-11], antiviral [12] activities.

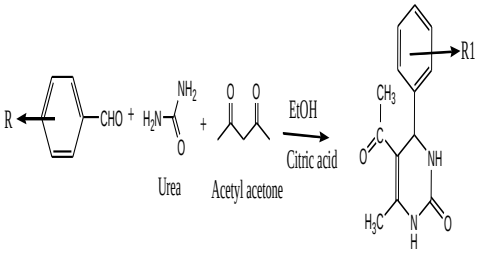
Biginelli reaction is the classical example for single pot multi-component reaction involving cyclocondensation of an aldehyde, active α-methelene group containg compounds (β-keto ester, acetyl acetone..,etc.) and urea in presence of lewis and organic acid. Claisen- Schmidt condensation reaction is generally used for the synthesis of chalcones by condensing the aromatic ketones and aldehydes in presence of base or acid.

MATERIALS AND METHODS:

All the chemicals used in the synthesis were procured from Sigma Aldrich. TLC plates for monitoring of reactions and silica gel (100-200 mesh) for column chromatography obtained from Merck. The Melting points were determined by using EZ-Melt automated melting point apparatus. The FT-IR spectra were recorded on BRUKER ALPHA-T FT.IR spectrophotometer using KBr pellet method. The Mass spectra were recorded on Agilent 6320 Ion Trap LC-MS (Positive/Negative ion electro spray ionization method). The 1H NMR spectra of the compounds were recorded on BRUKER 400 MHz NMR Spectophotometer.

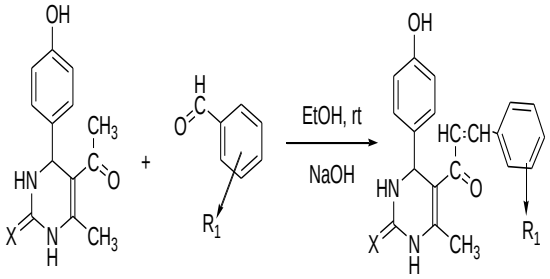
EXPERIMENTAL:

Step: 1 synthesis of Dihydro pyrimidinone derivatives: A mixture of Aldehyde (10millimoles(mmol)), acetylacetone (11mmol), urea (13mmol) and citric acid (5mmol) were taken in a round bottomed flask and refluxed for 6-12 hours at 80^0C. After completion of reaction is monitored by TLC, obtained crude product was transferred into a beaker containing crushed ice, filtered, washed with ice cold water and recrystallized using ethanol. spectral data of synthesied compound(1c). IR: 1688.73(C=O), 3333. 80(NH), 1124.11(OC_2H_5), 1240.38(C-N) NMR: 1.34(3H,CH3); 2.06 (s, 3H,CH3); 2.27 (s, 3H,COCH3); 4.01(2H, OCH2);5.18 (s, 1H, C-H); 6.83(s, 1H Ar – H);6.62(1H,Ar-H); 6.60(1H, Ar-H);9.09(s, 1H,CONH); 8.85 (s, 1H,OH);7. 69 (1, NH), Mass: Mass(m/z) (M+1)



Step: 2 syntheses of chalcones of dihydropyrimidinones (2a-2e):

A mixture of compound j(1mmol), aldehydes(1mmol) and 20ml ethanol(to soluble the contents) were taken in a beaker and to that add 10 ml of 30% aqueous solution of NaOH and then subjected to stirring up to 48 hours reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured on to ice cold water and acidified with dil.HCl. Then obtained solid was filtered, washed with water and obtained products were purified by column chromatography. Synthesized chalcones and their physicochemical properties were listed in table no.3, 4. Spectral data(2d): IR :1688.73(C=O), 3333. 80(NH), 1122.11(C-Cl), 1607.42(C=C), 1240.38(C-N) NMR: 1.34(3H,CH3); 2.06 (3H,CH3 S); 2.27 (3H,COCH3 S); 4.01(2H, OCH2);5.18 (1H, C-H of pyrimidine ring); 6.83(d,1H,J=17.5CH==CH);6.62(d,1H,J=17.6 CH==CH); 7.60(m,5HAr-);9.09(1H,CONH); 8.85 (1H,OH);7. 69 (1, NH), Mass: Mass(m/z) 395(M+1



ANTIBACTERIAL ACTIVITY:

Antimicrobial activity was determined by using agar cup against gram positive viz., *Staphylococcus areus* (NCIM 2079) and gram negative viz., *Escherichia coli* (NCIM 2068), gram positive plate method. The tested organism were sub cultured on nutrient agar medium, **Ampicillin** was used as reference drug for bacterial strain. The medium used for the antibacterial study has the following composition [g/L]: Peptone, Beef extract, sodium chloride; and agar in distilled water pH was adjusted at 7. Bacterial culture was incubated at 37° C for 24hours.The solution of the test compounds were prepared by dissolving 0.5mg/1ml and 1mg/10ml of dimethylsulfoxide at a concentration of 100µg/50µl and 250µg/50µl. The cups each of 10 in diameter were made by scooping out medium with a sterilized cork borer in a Petri. The solutions of each test compound (50 µl) were added separately in the cups and petri dishes were subsequently incubated at 37 0C for 24 hours. Zone of inhibition produced by each compound was measured in millimeters (mm). Compounds were screened for their antibacterial activity (table no.5).

RESULTS AND DISCUSSIONS:

All the dihydropyrimidinonene derivatives (1a-1j) and their chalcones (2a-2e) were evaluated for their antibacterial against both grampositive *(Staphylococcus areus)* and gramnegative (E.coli), by using cup plate method. The results of antibacterial activity were given in table no: interestingly all the synthesized dihydropyrimidinone derivatives were shown mild to moderate antibacterial activity at 50µg/ml and 100µg/ml concentration , when compared with the standard drug (Ampicillin) at same concentrations . Compounds **1c, 1e, 1i, 1j, 2c** and **2d** were showed potent antibacterial activity against both gram positive and gram negative organisms.

ANTIFUNGAL ACTIVITY:

Antifungal activity was determined by using agar cup plate method. The tested organism *Aspergillus niger* (ATCC 6275) was sub cultured on potato-dextrose agar (PAD) for fungi. Fluconazole was used as positive control for fungi. Fungal culture was incubated at 25-30 0C for 4-5 days. .The solution of the test compounds were prepared by dissolving 5mg/10ml and 10/10ml of dimethylsulfoxide at a concentration of 50µg/1ml and 100µg/1ml. The cups each of 10 in diameter were made by scooping out medium with a sterilized cork borer in a Petri dish. The solutions of each test compound (50µL) were added separately in the cups and petri dishes were subsequently incubated incubated at 25-30 0C for 4-5 days. Zone of inhibition produced by each compound was measured in millimeters (mm). Compounds were screened for their antifungal activity (table no.6)

RESULTS AND DISCUSSIONS: All the dihydropyrimidinonone derivatives (1a-1j) and their chalcones (2a-2e) were evaluated for their antifungal acticity against *Aspargillus niger*, by using cup plate method. The results of antifungal activity were given in table no: it is noticed that all the synthesized dihydropyrimidinone derivatives were shown mild to moderate antifungal activity at 50µg/ml and 100µg/ml concentration , when compared with the standard drug (Fluconazole) at same concentrations . Compounds **1c, 1i, 1j, 2b** and **2d** were showed better antifungal activity.

Table no: 1: Structures of dihydropyrimidinoes (1a-1j).

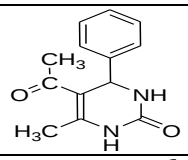
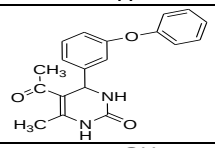
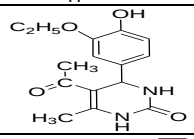
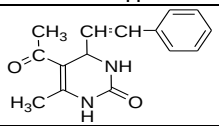
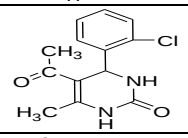
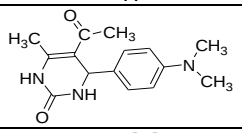
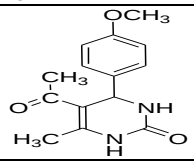
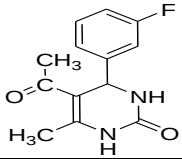
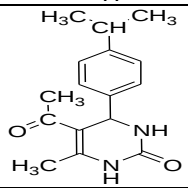
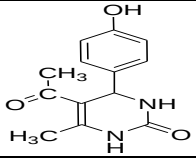
S.no	compound	structure	IUPAC name
1	a		5-acetyl-4-phenyl-3,4-dihydro-6-methylpyrimidine-2[1H]-one
2	b		5-acetyl-4-(3-phenoxy phenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one
3	c		5-acetyl-4-(3-ethoxy-4-hydroxy phenyl-3,4-dihydro-6-methylpyrimidine-2[1H]-one
4	d		5-acetyl-4-styryl-3,4-dihydro-6-methylpyrimidine-2[1H]-one
5	e		5-acetyl-4-(2-chlorophenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one
6	f		5-acetyl-4-(4-N,Ndimethylphenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one
7	g		5-acetyl-4-(4-methoxy phenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one
8	h		5-acetyl-4-(3-fluro phenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one
9	i		5-acetyl-4-phenyl-3,4-dihydro-6-methylpyrimidine-2[1H]-one
10	j		5-acetyl-4-(4-hydroxyphenyl)-3,4-dihydro-6-methylpyrimidine-2[1H]-one

Table no: 2-Physicochemical properties of dihydropyrimidinones (1a-1j)

S.No	compound	R	Molecular formula	Molecular weight	Yield (%)	Melting point (^{0}C)
1	a	H	$C_{14}H1_5N_2O_2$	243	81	210
2	b	O-C_6H_5	$C1_9H_{18}N_2O_3$	322	78	195
3	c	3-OC2H5, 4-OH	$C_{15}H_{18}N_2O_3$	290	80	205
4	d	CH=CH-	$C_{15}H_{16}N_2O_2$	256	79	201
5	e	2-Cl	$C_{13}H_{13}N_2O_2Cl$	264.5	80	204
6	f	N-$(CH_3)_2$	$C_{15}H_{18}N_3O_2$	278	68	200
7	g	OCH_3	$C_{15}H_{18}N_2O_3$	274	83	190
8	h	3-F	$C_{13}H_{13}N_2O_2F$	247.9	80	206
9	i	CH-$(CH_3)_2$	$C_{16}H_{20}N_2O_2$	272	82	198
10	j	P-OH	$C_{14}H_{16}N_2O_3$	260	85	215

Table no: 3- Structures of chalcones (2a-2e)

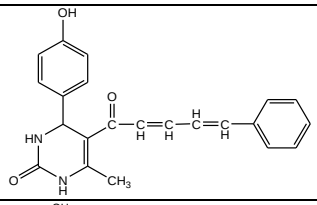
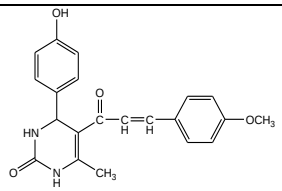
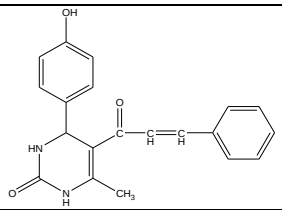
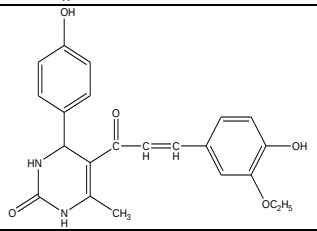
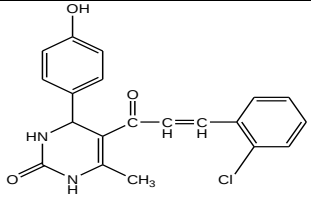
s.no	Compound code	Structures	IUPAC name
11	2a		5-(5-phenylpenta-2,4-dienoyl)-3,4-dihydro-4-(4-hydroxyphenyl)-6-methylpyrimidin-2(1*H*)-one
12	2b		3,4-dihydro-4-(4-hydroxyphenyl)-5-(3-(4-methoxyphenyl)acryloyl)-6-methylpyrimidin-2(1*H*)-one
13	2c		3,4-dihydro-4-(4-hydroxyphenyl)-6-methyl-5-(3-phenylacryloyl)pyrimidin-2(1*H*)-one
14	2d		5-(3-(3-ethoxy-4-hydroxyphenyl)acryloyl) -3,4-dihydro-4-(4-hydroxyphenyl) -6-methylpyrimidin-2(1*H*)-one
15	2e		5-(3-(2-chlorophenyl)acryloyl)-3,4-dihydro -4-(4-hydroxyphenyl)-6-methylpyrimidin-2(1*H*)-one

Table no: 4- Physicochemical properties of chalcones (2a-2e).

code	Molecular formula	Molecular weight	Melting point (^{o}c)	Colour	Percentage yield
2a	$C_{22}H_{20}N_2O_3$	360.41	206	yellow	75
2b	$C_{21}H_{20}N_2O_4$	364.39	209	yellow	78
2c	$C_{20}H_{18}N_2O_3$	334.37	201	brown	70
2d	$C_{22}H_{22}N_2O_5$	394.42	205	brown	77
2e	$C_{20}H_{17}ClN_2O_3$	368.81	210	orange	80

Table no: 5-Anti bacterial activity results:

S.NO	COMPOUNDS	**Zone of inhibition(mm)**			
		Staphylococcus aureus		***Escherichia coli***	
		50µg/ml	100 µg/ml	50µg/ml	100µg/ml
1	1a	9	10	12	13
2	1b	10	11	-	10
3	1c	11	16	13	16
4	1d	13	14	11	15
5	1e	11	**17**	15	16
6	1f	11	13	11	14
7	1g	12	14	12	**17**
8	1h	13	**17**	15	**17**
9	1i	12	14	13	14
10	1j	13	**17**	10	16
11	2a	14	17	15	16
12	2b	15	17	16	17
13	2c	15	**18**	16	**19**
14	2d	15	**18**	15	**19**
15	2e	14	17	14	17
16	**Ampicillin**	**17**	**19**	**19**	**21**

Table no: 6-Anti fungal activity results:

S.NO	COMPOUNDS	Zone of inhibition(mm)	
		Asparagillus niger	
		50 µg/ml	100 µg/ml
1	1a	13	15
2	1b	14	16
3	1c	16	**20**
4	1d	16	18
5	1e	14	16
6	1f	15	18
7	1g	15	17
8	1h	15	**20**
9	1i	15	16
10	1j	14	18
11	2a	17	19
12	2b	17	**23**
13	2c	16	20
14	2d	17	**22**
15	2e	17	20
16	**Fluconozole**	**22**	**25**

CONCLUSION:

In present we successfully synthesized dihydropyrimidinones were by condensation of urea and acetylacetone with different aldehydes in presence of citric acid with good yield and characterized by IR, NMR, and MAS spectral studies. Chalcones of compound (1j) synthesized by condensation with different aldehydes in presence 30% NaOH and characterized by spectral studies. All the compounds were evaluated for antibacterial and antifungal activity. Compounds **1c, 1e, 1i, 1j, 2c** and **2d** were showed potent antibacterial activity against both gram positive and gram negative organisms. Compounds **1c, 1i, 1j,2b** and **2d** were showed poent antifungal activity. Compounds those contain electron withdrawing group such as halogens showed better anti bacterial and compounds containing electron releasing groups showed better antifungal activity .Further research is in progress which may bring some productive results. It is hoped that pyrimidinone derivatives could prove to be valuable scaffolds for future development of more potent biologically active agents through structural modification and derivatization.

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