Research Article Rani *et al.*, 9(4): April, 2018:5779-5784]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Conventional synthesis, characterization and *In-vitro* evaluation of 4, 6-disubstituted pyrimidine-2-one derivatives

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Abstract

A Series of Bioactive compounds 4,6 –Di Phenyl Pyrimidine-2-ol(4a) , 4-(3- nitro phenyl) -6- Phenyl - 2(1H)-Pyrimidinone (4b), 4-(2-hydroxy phenyl)-6- phenyl pyrimidin-2(1H)- one(4c), 4,[4- (Dimethyl amino) phenyl]-6-Phenyl Pyrimidine -2(1H)-one(4d), 4-(2-chlorophenyl) -6- Phenyl Pyrimidine- 2(1H)-one(4e), were Synthesized according to the Literature methods. The Synthesized compounds were characterized by NMR, IR & Mass Spectroscopy. All the compounds have been evaluated for invitro antimicrobial activity and were compared with their corresponding standards. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of 4,6- Disubstituted Pyrimidine-2 -one derivatives on some bacterial (Two gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis* and One Gram-negative bacteria *Pseudomonas aeruginosa)* and fungal strains (Three different fungal strains *Aspergillus niger, Pencillium chrysogenum* and *Pencillium notatum*. Melting points were determined in open glass capillaries using GallenKamp (MFB-600) melting point apparatus and were uncorrected. IR spectra (KBr discs) Bruker analyzers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, Model No.8400S (Japan). 1H and 13C NMR spectra were recorded on Bruker 400 MHz NMR spectrometer (Switzerland) using DMSO as solvent. T.L.C. was run on silica gel G plates using ethyl acetate: n-hexane (7:3) as developing solvent to assess the progress of reaction and purity of the compounds. All other chemicals used in the present study were of analytical grade. All the compounds showed significant antibacterial and anti-fungal activity but more active towards gram positive bacteria of all the derivatives synthesized compounds 4b, 4d exhibited good and compounds 4a, 4c, 4e showed moderate antimicrobial properties.

Key Words: 4, 6- Disubstituted Pyrimidine-2- one, Anti- bacterial, Anti-fungal, Heterocyclic Chalcones.

Introduction

Out of Heterocyclic compounds, pyrimidines derivatives have been very well known for their therapeutic applications in medicinal chemistry. Pyrimidine and their derivatives play the vital role in DNA & RNA; it is associated with various biological activities [1]. Pyrimidine derivatives are interest due to their pharmacological properties such as anti tumour[2-5], Antiviral[6], antifungal, anti cancer[7], antibacterial[8], anti inflammatory[9-12], analgesic[13], antagonist[14,15], anti-folate[16], anti microbial[17], anti HIV[18], Anti Proliferative[19], Anti-platelet[20], Antithrombotic[21], Anti-filarial[21], activities etc.

An Anti-Microbial is agents that kills Micro-

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Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Hence the Titled compounds are evaluated for anti-bacterial activity.

Fungal infections cause one million deaths per year worldwide. The problem is many of the antifungal drugs doctors have relied on for years are becoming less and less effective as these infections build up antifungal resistance. That's why we need to expand the range of antifungal drugs doctors have at their

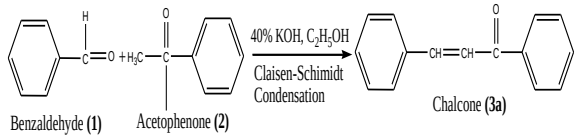
disposal and there is a need to investigate newer anti-fungal drugs. Hence the Titled compounds are evaluated for anti-fungal activity.

Drugs and chemicals

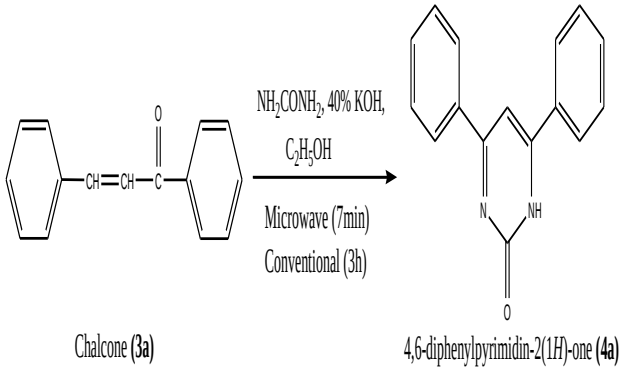
Benzaldehyde-(MERCK-B.No:SC1S610109), O-HydroxyBenzaldehyde-(MERCK-B.No: PC/201/16-2), 2-Chloro Benzaldehyde-(PALLAV-B.No:PC/388-2/17-2), m-Nitro Benzaldehyde-(PALLAV-B.No: PC/2186/16-2),4-Methyl amino benzaldehyde MERCK-B.No:QD5Q650881), Acetophenone-(FINAR-B.No:5065602212BP),Ethanol-(CSS.B.No-110605), Potassium Hydroxide-(FISHER), Silica gel-G-(RSEARCH-LAB FINE CHEM industries-B.No:1317310113), Ethyl acetate-(AVRA), Dimethyl Sulphoxide-(AVRA-B.No:N140122180), Sodium Chloride-(FINAR-B.No:76274020).

General procedure for synthesis of 4, 6-Disubstituted pyrimidine-2-one Derivatives (4a-4e):

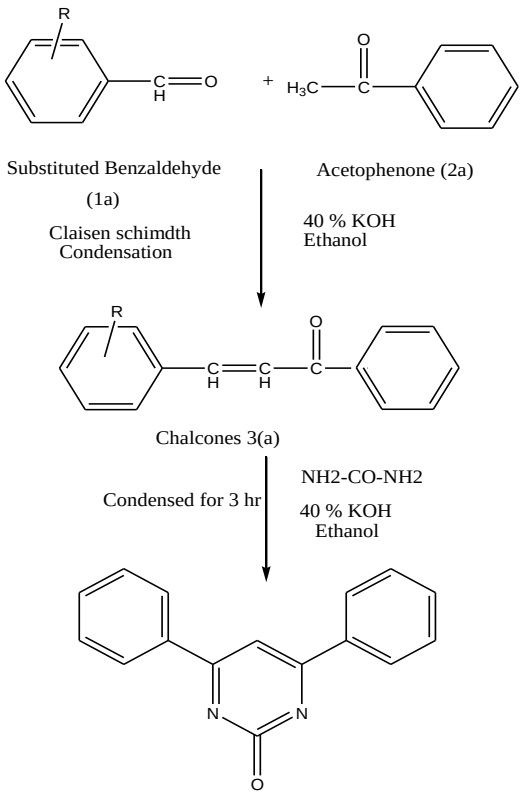
Step-1: Synthesis of Chalcones: Acetophenone (0.01mol, 1.2g, Benzaldehyde (0.01mol, 1.06g) were mixed and dissolved in Ethanol (10mL). To this aqueous potassium hydroxide solution (10 ml) was added slowly With constant stirring. The reaction mixture was stirred continuously for 3h at room Temperature. The completion of reaction was confirmed by monitoring TLC using silica gel- G. After completion of the reaction, the reaction mixture was kept in refrigerator Overnight. The product was filtered and washed with cold water till the washings were neutral to Litmus, if necessary acidified with dilute HCl. The product was dried and recrystallized from Rectified spirit to get pale yellow coloured solid chalcones.



Step-2; synthesis of 4, 6-diphenylpyrimidin-2(1H)-one (4a): Conventional Synthesis: Chalcones (0.01mol, 2.08g), urea (0.01mol,0.6g) were mixed and dissolved in ethanol (10ml). To this 40% aqueous potassium hydroxide solution 10ml was added slowly with constant stirring. The Reaction Mixer was refluxed on water bath for 3h. In between TLC was monitored to check the completion of reaction. After completion of reaction, the reaction mixture was cooled to room temperature. And then poured in to ice cold water and neutralized by adding dilute HCl. The Precipitate obtained was filtered, washed with water and dried. The product was recrystallized from rectified spirit. The procedure was illustrated under **Scheme 1.**



SCHEME:



Pyrimidine-2- one derivatives (4a-e)

***In vitro* Studies:**

Antibacterial activity [23]: All the synthesized compounds 4a-4e were examined for *invitro* antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureusNCIM2901, Bacillus subtilis MTCC 441* and one Gram-negative bacteria *Pseudomonas aeruginosa* by diffusion method. Tetracycline and Chloramphenicol were used as an internal standard.

Nutrient agar (High media) was dissolved and distributed in 25ml quantities in boiling tubes and were sterilized in an autoclave at 121^0C (15lbs / sq.in) for 15minutes.The medium was inoculated at one percent level using 18 hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for above 30min.In a size of 4 inches petridishes, five cups of 8mm diameter at equal distance were made in each plate. In the cups the test solutions of different concentrations were added and in another plate cups were made for standard and control. The plates thus prepared were left for 60 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37^0C the plates were examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition measured and recorded. The results were represented in **Table 6**.

Antifungal activity [24] : The antifungal activity of compounds was assayed against three different fungal strains *Aspergillus niger MTCC 282, Pencillium chrysogenum MTCC5108* and *Pencillium notatum NCIM 742.*

Potato dextrose agar (Hi- media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and was sterilized in an autoclave at 121 °C (15lbs/sq.in) for 15 minutes. The medium was inoculated with 1% 18hr old cultures of organisms aseptically in to sterile petridishand allowed to set at room temperature for about 30 minutes. At a size of 4 inches petridish 5 cups of 8mm diameter at equal distance were made in a petriplates with a sterile borer. The solutions of test concentrations (250µg/ml, 200µg/ml, 150µg/ml and 100µg/ml) and standard were added to respective cups aseptically and labelled accordingly.DMF as control did not show any inhibition. The plates were left for 90 minutes in refrigerator for diffusion and incubated for 72 hrs at $37^0 \pm 1^0$c. The plates were examined for inhibition zones. Fluconazole was used as standard. The experiments were performed in duplicate and the average diameters of the zones of inhibitions were summarized in **Table 7.**

Result and Discussion

The Physical Data of synthesized compounds were tabulated in **Table 1&2.** The synthesised compounds were established through IR, 1H and 13C NMR spectral data. The IR spectra of (4a-4e) exhibited absorption bands for imines (–C=N–) at 1541 cm-1, imines (–C=O–) at 1711 cm-1, alkenes (–C=C–) 1651 cm-1, Nitro group ($–N0_2$) at 1510 cm-1, Hydroxy group (-OH) at 3753 cm-1, Chlorine (-C—Cl) at 775 cm-1, alkanes (-C-H) at 1305 cm-1. The $1H^{NMR}$ spectra of these compounds revealed signals at δ = 7.14-8.6 ppm a multiplet for aromatic protons, δ = 8.0 ppm a double for(C=N), δ = 4.9 ppm a singlet for (CH=C). The 13C NMR spectra of these compounds revealed signals at δ = 133-164 ppm peaks for aromatic carbons , δ = 106-135 ppm for(C-H), δ = 164 ppm for(N=C), δ = 133 ppm for alkene carbons (–C=C-), δ = 156 ppm for carbonyl carbon (-C=O), δ = 156 ppm for(O=C-NH), δ = 75 ppm for($Ar-NO_2$), δ = 157 ppm for(C-OH), δ = 40 ppm for($N-CH_3$), δ = 70 ppm for(C-Cl). The structural characterisations of synthesised compounds were done and were tabulated in **Tables 3, 4 and 5.**

Table 1: Synthesized compounds

COMPOUND	4a	4b	4c	4d	4e
R	-H	-NO2	-OH	-N(CH3)2	-Cl

Table 2: Physical Data

Cpd	Compound	M.F	M.W	MP (^{0}C)	% Yield	C%	H%	O%	N%	Cl%
4a	4,6 –Diphenyl Pyrimidine -2-ol	$C_{16}H_{12}N_2O$	248	90-93	58.67	77.40	4.87	6.44	11.28	–
4b	4–(3-Nitrophenyl)–6-Phenyl 2(1H)- Pyrimidinone	$C_{16}H_{11}N_3O_3$	293	176-179	62.85	65.53	3.78	16.37	14.33	–

4c	4-(2-Hydoxyphenyl)-6-phenyl- pyrimidin-2(1H)-one	$C_{16}H_{12}N_2O_2$	264	185-190	62.56	72.72	4.58	12.11	10.60	_
4d	4,[4(Dimethylamino)phenyl]-6- phenyl pyrimidin-2(1H)-one	$C_{18}H_{17}N_3O$	291	250-252	57.78	74.20	5.88	5.49	14.42	_
4e	4-(2-Chloophenyl)-6- phenyl Pyrimidin -2(1H)-one	$C_{16}H_{11}ClN_2O$	282	185-190	63.59	67.97	3.92	5.66	9.91	12.54

Table 3: IR Data

Compound	-C=C	=C-H	C=O	C=N-	-C-N	NO2	O-H	C-Cl
4a	1651	3032	1711	1541	1305	-	-	-
4b	1651	3032	1711	1541	1305	1510	-	-
4c	1651	3032	1711	1541	1305	-	3743	-
4d	1651	3032	1711	1541	1305	-	-	-
4e	1651	3032	1711	1541	1305	-	-	775

Table 4: ^{13}C NMR

Cpd	C-H	C	C=O	C=C	C=N	O=C-NH	Ar-NO2	C-OH	N-CH3	C-Cl
4a	106,126,128,129,131.	133,134,154,163,164.	156	133	164	156				
4b	106,122,126,128,129,135.	134,156,163,164.	156	133	164	156	75			
4c	106,115,118,121,126,128,130.	134,156,158,163,164.	156	133	164	156		157		
4d	106,111,116,118,126,128,129.	134,149,156,163,164.	156	133	164	156			40,40	
4e	106,126,127,128,129,130,131.	134,156,163,164.	156	133	164	156				70

Table 5: ^{1}H NMR

Compound	Hydrogen (n)	δ (ppm)	Multiplicity	Solvent
4a	a. Vinylic protons(-C**H**=C) (SP2), b. Aromatic protons(Ar-H), c. HC=N	4.9 7.14—7.6 8.0	Singlet Multiplet Doublet	DMSO
4b	a. Vinylic protons (-C**H**=C) (SP2), b. Aromatic protons(Ar-H), c. HC=N	4.9 7.14-8.6 8.0	Singlet Multiplet Doublet	DMSO
4c	a. Vinylic protons(-C**H**=C) (SP2), Aromatic protons(C-OH) b. Aromatic protons(Ar-H), c. HC=N	4.9,5.0 6.8-7.3 8.0	Singlet Multiplet Doublet	DMSO
4d	a. Vinylic protons(-C**H**=C) (SP2), b. Aromatic protons(Ar-H), c. HC=N	4.9, 2.85 6.6-7.3 8.0	Singlet Multiplet Doublet	DMSO
4e	a. Vinylic protons (-C**H**=C) (SP2), b. Aromatic protons(Ar-H), c. HC=N	4.9 7.14-7.6 8.0	Singlet Multiplet Doublet	DMSO

All the compounds **(4a-4e)** were tested for antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureus NCIM 2901, Bacillus subtilis MTCC 441* and one gram-negative bacteria *Pseudomonas aeruginosa*.Tetracycline and Chloramphenicol were used as standards. For antifungal activity against three fungal strains *Aspergillus niger MTCC 282, Pencillium chrysogenum MTCC5108* and *Pencillium notatum NCIM 742*. Fluconazole was used as standard.

The results of antimicrobial activities of synthesized compounds were shown in **Table 6 and 7**. All the compounds showed significant antibacterial and antifungal activity but more active towards gram positive bacteria of all the derivatives synthesized compounds **4b, 4d** exhibited good and compounds **4a, 4c, 4e** showed moderate antimicrobial properties.

The structure-activity relationship studies based on the above *in vitro* results clearly indicate that compounds with moderate electron donating groups mainly mono and dialkyl substituted amino group on the aromatic ring showed increased potency when compared to the strong electron donating groups such as hydroxy. The presence of halo groups mainly chlorine also showed good activity. The intense activity of the compounds is also greatly influenced by the position of the groups on the ring. The results also indicate the influence of rise in activity with increase in the number of alkyl group mainly methyl substituent.

Table 6: Antibacterial activity:

Compound	Bacillus subtilis				Staphylococcus aureus				Pseudomonas aeruginosa			
	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
4a	0.9	1.1	1.3	1.4	1.1	1.2	1.3	1.4	1	1.1	1.2	1.4
4b	1	1.2	1.4	1.5	1.1	1.2	1.2	1.4	1.1	1.3	1.4	1.6
4c	1.1	1.2	1.3	1.5	1.1	1.2	1.3	1.4	1.1	1.1	1.2	1.3
4d	1.2	1.3	1.4	1.6	1.5	1.5	1.6	1.7	1.2	1.4	1.6	1.7
4e	1.2	1.3	1.3	1.4	1.1	1.1	1.2	1.3	1.1	1.2	1.3	1.5
Tetracycline	2	2	2.1	2.2	2.1	2.2	2.3	2.3	2	2.1	2.1	2.2
Chloramphenicol	2.1	2.2	2.3	2.4	2	2.1	2.2	2.3	2.1	2.2	2.3	2.4

Table 7: **Antifungal Activity**

Zone of inhibition (Diameter In Cm)												
Compound	**Aspergillums Niger**				**Pencillium chrysogenum**				**Pencillium notatum**			
	100 µ/ml	**150µ/ ml**	**200 µ/ml**	**250 µ/ml**	**100 µ/ml**	**150 µ/ml**	**200 µ/ml**	**250 µ/ml**	**100 µ/ml**	**150 µ/ml**	**200 µ/ml**	**250 µ/ml**
4a	0.9	0.9	1	1.1	1	1	1.1	1.1	1.1	1.1	1.2	1.3
4b	1.1	1.3	1.4	1.5	1.2	1.3	1.4	1.9	1.1	1.4	1.5	1.7
4c	1	1.1	1.2	1.3	1	1.1	1.2	1.2	1	1	1.1	1.2
4d	1.1	1.1	1.2	1.3	1.2	1.3	1.3	1.4	1.5	1.6	2.1	2.2
4e	1.1	1.3	1.4	1.5	1.2	1.3	1.4	1.9	1.1	1.4	1.5	1.7
Fluconazole	1.7	1.8	1.9	2.0	1.6	1.8	1.9	2.0	1.8	1.9	2.0	2.1

Conclusion

A series of new **2, 4-Disubstituted -Pyrimidine-2-one derivatives (4a-4e)** were prepared by conventional method and evaluated for their *In-vitro* antimicrobial, ferric oxide reducing properties for which the mechanisms underlying this process remain to be fully elucidated. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an

approach for further optimization and development to get new leads in the treatment of microbial infections.

Acknowledgement

The authors are thankful to the Sri Siddhartha Pharmacy College for providing necessary facilities to carry out this research work.

References

[1]. K.M. Ghoneim, R.Youssef J Indian Chem soc, 53(1986), p.914.
[2]. N. Zhang, S.Ayral Kaloustial, T. Nguyen, R.Hernandezb, C.Beyerb Bioorg Med Lett 2007 Chem Lett17 (2007), p.3003.
[3]. N.R.Mohamud, M.M.Talaat EI- Saidi, Y.M.Alia, M.H.Elnagdi Bioorg Med Chem, 15 (2007), p.6227
[4]. E.M.Rakib, S.Abouricha, A.Hannioui, N.Benchat, L.Ait M 'barek, A.Zyad J Iranian, Chem Soc (3)(2006), p.272
[5]. P.G.Baraldi, M.G.Pavani, M.Nunez, *et al*. Med Chem Lett, 10(2002), p. 449
[6]. A.M.Kamela, B.Munsonb J Ame Soc Mass Spectr, 18 (2007),p. 1477
[7]. N. Zhang, S.Ayral-Kaloustiian, T. Nguyen, *et al*. J Med Chem 50 (2007),p. 319.
[8]. O.Prakash, R.Kumar, R.Kumar, P.Tyagi, R.C.Kuhad Euro J Med Chem, 42 (2007),p.868
[9]. S.M. Sondhi, S.Jain, M.Dinodia, R.Shuklab, R.Raghubir Bioorg Med Chem, 15 (2007),p. 3334
[10]. P.Molina, EAller, A.Lorenzo,*et al*. J Med Chem, 44(2001),p. 1011.
[11].S.S.Bahekar, D.B.Shinde Acta Pharm, 53 (2003), . 223.
[12]. S.M.Sondhi, M.Johaar, S.Rajvanshi, *et al*. Aust J Chem, 54 (2001),p.69.
[13]. O.Bruno, C.Brullo, S.Schenone, *et al*. Farmaco, 57 (2007),p.753.
[14]. M.C.Lanier, M.Feher, N.J.Ashweek, *et al*. Bioorg Med Che, 15 (2007),p.5590
[15]. N.J.Liverton, R.A. Bedner, B.Bedner, *et al*. S J Med Chem, 50, (2007),p.807.
[16]. A. Gangjee, Y.Zeng, J.J.McGuire, R.L.Kisliuk J Med Chem, 48 (2005),p.5329.
[17]. F.A.Eid, A.F.A.El-Wahab, G.A.M. El-Hag Ali, M.M.Khafagy Acta Pharm, 54 (2004), p.13
[18]. D.M.Coe, P,L.Meyers, D.M.Paryl J Chem Soc, 2 (1990),p. 157.
[19]. L.Xiaoling, G.Mei—Lin Bioorg Med Chem, 14 (2006),p.153.
[20]. O.Bruno, C.Brullo, S.Schenone, *et al*. Med Chem, 14 (2006),p.121.
[21]. S.B.Katiyar, I.Bansal, J.K.Saxena, P.M.S.Chauhan Bioorg Med Chem Lett, 15 (2005),P.47.
[22]."Antimicrobial". Merriam-Webster Online Dictionary. Archieved from the original on 24 April 2009. Retrieved 2009-05-02.
[23]. Naveen PV, Susmitha B, Jhansi G, Chaitanya G, Anupama B, Chenchu Lakshmi KNV. Synthesis and Anti-bacterial studies of new sulfonyl benzocoumarin derivatives. International Journal of Research in Pharmacy and Chemistry 2013; 3(4):808-812.
[24]. P.Pavan Kumar, S.Sridhar, K.Jagatheesh, E.Namasivayam. Synthesis and biological activity of imidazole derived chalcones and its pyrimidines. Int.J.Res. Ayurveda Pharma. 2013; 4(3):355-362.

How to cite this article

Sudha Rani K, Lakshmi Durga Jalasuthram, Srilatha Manikala, Sravani Mocharla, Sunand Variganji, Vinod Banavathu and T.Lakshmi (2018). Conventional synthesis, characterization and *In-vitro* evaluation of 4, 6-disubstituted pyrimidine-2-one derivatives. *Int. J. Pharm. Life Sci.*, 9(4):5779-5784.

Source of Support: Nil; Conflict of Interest: None declared

Received: 19.03.18; Revised: 22.03.18; Accepted: 27.04.18