International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Synthesis, characterization and biological evaluation of substituted pyrimidines.

Bhargav A[1], Narendra B. Gowda[2]* and Pavan kumar C[3]
[1,2,3] *Department of Pharmaceutical Chemistry, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore-560070, Karnataka, India.*

Date of Submission: 10-10-2021 Date of Acceptance: 22-10-2021

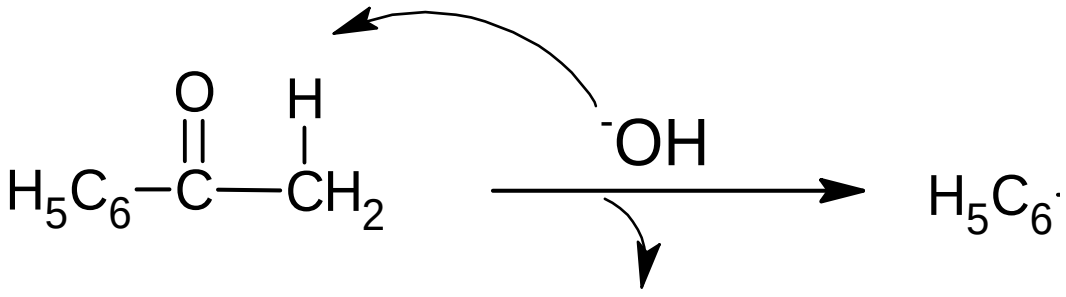
ABSTRACT: A series of substituted pyrimidines (SW-1A - SW-5A and SW-1B - SW-5B) synthesized from corresponding chalcones. Chalcones were prepared by Claisen schmidt condensation between p-chloroacetophenone and substituted benzaldehyde and chalcones were then cyclised with guanidine nitrate via Michael's addition to get substituted pyrimidines. Later the pyrimidines were acetylated and chloroacetylated to yield substituted pyrimidine derivatives. The chemical structures of synthesized compounds were confirmed by IR, NMR spectral studies and the synthesized compounds were screened for antibacterial and antifungal activities by cup-plate method.

KEYWORDS: Chalcones, Pyrimidines, Antibacterial and Antifungal.

I. INTRODUCTION:

The antimicrobial agents are the agents used to kill/inhibit the growth of microorganisms. Even the abundance of antimicrobial agents is more still, there is constant research is going on them because of the challenges like toxicity, hypersensitivity reactions, drug resistance and super infection that arise while using them[1,2]. So, in this research work, we attempted to synthesize pyrimidine derivatives for better antimicrobial activity i.e., antibacterial and antifungal activities.

Chalcones have many biological activities and can be used as precursors for the synthesis of many biologically active heterocyclic compounds like pyrimidines, pyrazoles, pyridines, etc., so this was the rationale behind synthesizing chalcones as scaffold. Chalcones can be prepared by Claisen schmidt condensation between an aldehyde and a ketone in the presence of a base to form α, β unsaturated carbonyl compounds called Chalcones [3,4] **(Fig no.1)**.



International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Pyrimidines are organic heterocyclic compounds that possess two nitrogen atoms in their ring system. These pyrimidines have wide occurrence in the nature majorly seen in RNA and DNA of living organisms and they have huge and wide therapeutic application in medical field like antibiotic, anticancer agent, antimalarial, analgesic, anti-inflammatory, antipyretic, anti-fungal, anti-histaminic, anti-viral, anti-diabetic, anti-convulsant, antioxidant and herbicidal as well [5]. The pyrimidines can be synthesized by condensing chalcones with guanidine nitrate in the presence of base via Michael's addition [6] **(Fig no. 2)**.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

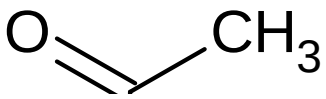
II. MATERIALS AND METHODS:

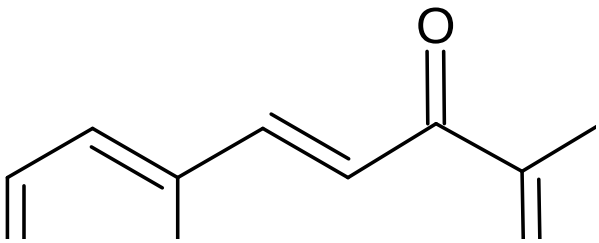
The chemicals required for the synthetic work were procured from SPECTROCHEM, CDH, and SDFCL. The purity of the compounds was checked by thin-layer chromatography (TLC) using silica gel G as stationary phase. The spots resolved were visualized by using UV and iodine chambers. The IR spectrum of the synthesized compounds was recorded on ATR-IR model Bruker alpha 2 in the ranges of 400-4000 and the values of Vmax were reported in cm^{-1}. ^{1}H-NMR spectra were recorded in Bruker 400MHz using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference tetramethylsilane (TMS). ^{13}C NMR spectra were recorded in Bruker 100 MHz using $CDCl_3$.

CHEMISTRY:

General procedure for the synthesis of 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one [7]:

p-chloroacetophenone (0.05 mol) was dissolved in 60 ml of ethanol in a round-bottomed flask stirred on a magnetic stirrer, 20 ml of 2% NaOH was added slowly. Immediately the reaction mixture turned golden yellow color. Then aromatic aldehyde (0.05 mol) was added drop wise through a dropping funnel. The stirring was continued at room temperature for about 3-4 hours. The precipitate obtained was filtered, washed with water, and recrystallized from ethanol.





International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Analytical data:

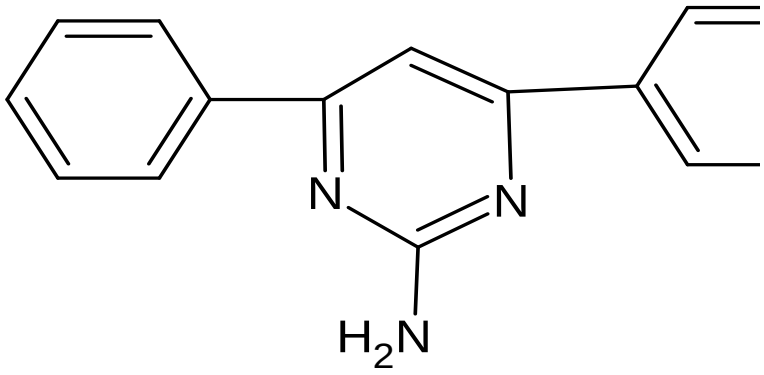
1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (A-1): Yield = 74%, m.p = 100-102 ºC, IR: 1658 (C=O), 1598 (C=C), 761 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98-7.95 (m, 2H, CH=CH), 7.82 (d,1H, Ar-H), 7.65-7.62 (m, 2H, Ar-H), 7.50-7.42 (m, 6H, Ar-H).

1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (A-2): Yield = 71%, m.p = 115 ºC, IR: 1654 (C=O), 1585 (C=C), 809 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97-7.95 (m, 2H, CH=CH), 7.79 (d, 1H, Ar-H), 7.60 (d, 2H, Ar-H), 7.49-7.45 (m, 2H, Ar-H), 7.36 (d, 1H, Ar-H), 6.94 (d, 2H, Ar-H), 3.86 (s, 3H, OCH_3).

1,3-bis(4-chlorophenyl)prop-2-en-1-one (A-3): Yield = 67%, m.p = 158 ºC, IR: 1651 (C=O), 1583 (C=C), 742 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.96-7.94 (m, 2H, CH=CH), 7.75 (d,1H, Ar-H), 7.58-7.37 (m, 7H, Ar-H).

General procedure for the synthesis of 4-(4-chlorophenyl)-6-phenylpyrimidin-2-amine [7]:

Chalcone (0.01 mol) and guanidine nitrate (0.01 mol) were taken in a three-necked flask, 30 ml of ethanol was added. The above mixture was refluxed, after the contents were dissolved in alcohol, an aqueous solution of NaOH (40%, 5ml) was added portion-wise for 3 hours. Reflux was continued further for 7 hours. The solvent was made to half of its volume and on cooling the solid product was separated. The obtained precipitate was washed with ice-cold ethanol and recrystallized from ethanol.



B-1

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Analytical data:

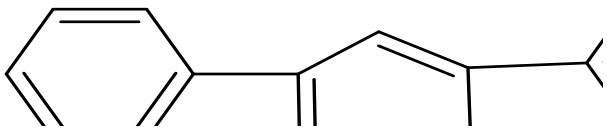
4-(4-chlorophenyl)-6-phenylpyrimidin-2-amine (B-1): Yield = 77%, m.p = 156 ºC, IR: 3304, 3182 (NH_2), 1630 (C=N), 767 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06-8.01 (m, 4H, Ar-H), 7.51-7.43 (m, 6H, Ar-H), 5.18 (s, 2H, NH_2).

4-(4-chlorophenyl)-6-(4-methoxyphenyl)pyrimidin-2-amine (B-2): Yield = 74%, m.p = 164 ºC, IR: 3324, 3202 (NH_2), 2835 (OCH_3), 1641 (C=N), 810 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05-7.98 (m, 4H, Ar-H), 7.45 (dd, 2H, Ar-H), 7.37 (s, 1H, Ar-H), 7.02-6.99 (m, 2H, Ar-H), 5.16 (s, 2H, NH_2), 3.88 (s, 3H, OCH_3).

4,6-bis(4-chlorophenyl)pyrimidin-2-amine (B-3): Yield = 63%, m.p = 214 ºC, IR: 3305, 3186 (NH_2), 1638 (C=N), 804 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.01 (m, 4H, Ar-H), 7.48-7.39 (m, 5H, Ar-H), 5.18 (s, 2H, NH_2).

General procedure for the synthesis of N-(4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl)acetamide:

Substituted pyrimidine (0.01 mol), glacial acetic acid (5ml), and acetic anhydride (0.02 mol) were taken in a round-bottomed flask and refluxed for 2 hours. After completion of the reaction, the reaction mixture was cooled and poured into a beaker containing cold water. The solid product obtained was filtered, washed with water, and recrystallized from ethanol.



International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Analytical data:

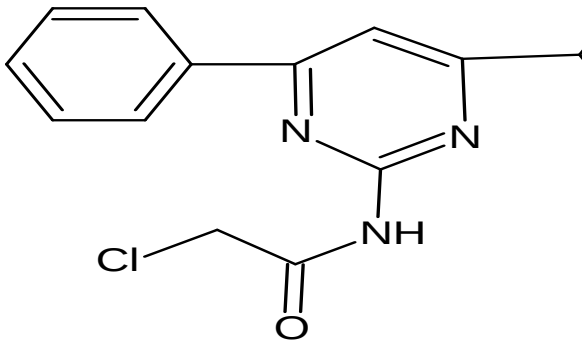
N-(4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl)acetamide (SW-1A): Yield = 77%, m.p = 180 ºC, IR: 3201 (NH), 1667 (C=O), 764 (C-Cl), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12-8.06(m, 5H, NH, Ar-H), 7.78 (s, 1H, Ar-H), 7.55-7.49 (m, 5H, Ar-H), 2.73 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.9, 164.4, 157.9, 137.5, 136.5, 135.0, 131.3, 129.2, 129.0, 128.5, 127.2, 107.2, 25.6.

N-(4-(4-chlorophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)acetamide (SW-2A): Yield = 73%, m.p = 204 ºC, IR: 3270 (NH), 1661 (C=O), 827 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.11-8.03 (m, 5H, NH, Ar-H), 7.70 (s, 1H, Ar-H), 7.51-7.47(m, 2H, Ar-H), 7.05-7.02(m, 2H, Ar-H), 3.89 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.7, 164.4, 162.3, 157.7, 137.2, 135.2, 129.1, 128.8, 128.4, 114.3, 106.2, 55.4, 25.6.

N-(4,6-bis(4-chlorophenyl)pyrimidin-2-yl)acetamide (SW-3A): Yield = 61%, m.p = 240 ºC, IR: 3406 (NH), 1665 (C=O), 827 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.11-7.99 (m, 5H, NH, Ar-H), 7.73 (s, 1H, Ar-H), 7.54-7.48 (m, 4H, Ar-H), 2.70 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): 165.1, 157.9, 137.6, 134.9, 129.6, 129.3, 128.9, 128.5, 106.9, 25.6.

General Procedure for synthesis for 2-chloro-N-(4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl)acetamide:

Benzene (30 ml), chloroacetylchloride (0.01 mol) and triethylamine (2ml) were taken in a three-necked flask, and the mixture was stirred on a water bath for 10 minutes, the solution of substituted pyrimidine (0.01 mol) in benzene (80 ml) was added drop wise and refluxed for 4 hours. Then the reaction mixture was cooled. The product was obtained in the form of crystals, filtered and washed with benzene, and recrystallized from ethanol.



International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Analytical data:

2-chloro-N-(4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl)acetamide (SW-1B): Yield = 70%, m.p = 172 ºC, IR: 3225 (NH), 1682 (C=O), 764 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.05 (s, 1H, NH), 8.20-7.48 (m, 10H, Ar-H), 4.69 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.5, 157.0, 137.8, 136.0, 134.6, 131.6, 129.9, 128.1, 127.3, 108.0, 103.1, 44.3.

2-chloro-N-(4-(4-chlorophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)acetamide (SW-2B): Yield = 68%, m.p = 256 ºC, IR: 3371 (NH), 2833 (OCH_3) 1678 (C=O), 781 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.60 (s, 1H, NH), 8.12-8.06 (m, 4H, Ar-H), 7.76 (s, 1H, Ar-H), 7.52-7.49 (d,d, 2H, Ar-H), 7.05-7.03 (d,d, 2H, Ar-H), 4.71 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.1, 150.9, 133.6, 132.5, 129.4, 129.1, 128.6, 114.6, 107.1, 55.6, 44.37.

N-(4,6-bis(4-chlorophenyl)pyrimidin-2-yl)-2-chloroacetamide (SW-3B): Yield = 58%, m.p = 179 ºC, IR: 3374 (NH), 1676 (C=O), 820 (C-Cl). ^{1}H NMR, ($CDCl_3$, 400 MHz): δ 8.65 (s, 1H, NH), 8.11-7.50 (m, 9H, Ar-H), 4.65 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.5, 161.7, 151.8, 134.3, 131.4, 129.5, 129.2, 128.7, 128.6, 46.4.

BIOLOGICAL EVALUATION:

Cup plate method [8,9] using nutrient agar medium was used to study the antibacterial activity of synthesized compounds against Staphylococcus aureus, Enterococcus faecalis (gram positive) and Escherichia coli, Klebsiella pneumonia (gram negative). Preparation of nutrient broth, subculture, inoculation, incubation done as per the standard procedure. Each synthesized compound (1mg) was dissolved in 1 ml of dimethyl formamide (1000 µg/ml). Volumes of 100 µg/ml and 200 µg/ml of each compound were used for testing.

Cup plate method using sabouraud dextrose agar to study the antifungal activity of synthesized compounds against Candida albicans and Aspergillus niger. Preparation of nutrient broth, subculture, inoculation, incubation done as per the standard procedure. Each synthesized compound (1mg) was dissolved in 1 ml of dimethyl formamide (1000 µg/ml). Volumes of 100 µg/ml and 200 µg/ml of each compound were used for testing.

The cups each of 9 mm diameter were made in the medium with a sterilized borer in a petridish, which was streaked with the organisms. The solutions of each test compound (100 µL and 200 µL) were added separately in the cups and petridishes were incubated. Trimethoprim and cotrimazole were used as reference drugs and dimethyl formamide used as a control which did not show any inhibition. Zone of inhibition produced by each compound was measured and the results are represented in **Tables 1 and 2.**

Table 1: Antibacterial evaluation of synthesized pyrimidine derivatives

Compound code	Zone of inhibition (mm)							
	Staphylococcus aureus		Escherichia Coli		Klebsiella pneumoniae		Enterococcus faecalis	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
SW-1A	-	16	-	14	-	18	-	20
SW-2A	-	16	-	16	-	16	-	16
SW-3A	-	12	-	12	-	16	-	18
SW-4A	-	14	-	12	-	16	-	14
SW-5A	-	12	-	12	-	14	-	12
SW-1B	-	14	-	14	12	16	-	20
SW-2B	-	12	-	14	16	18	-	18
SW-3B	-	12	-	14	12	16	-	16
SW-4B	-	14	-	10	12	18	-	18
SW-5B	-	14	-	12	14	18	-	18
Standard 50 µg/ml	18		16		30		25	
Control	-		-		-		-	

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 954-962 www.ijprajournal.com ISSN: 2249-7781

Table 2: Antifungal evaluation of synthesized pyrimidine derivatives

Compound code	Zone of inhibition (mm)			
	Candida albicans		Aspergillus niger	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
SW-1A	-	16	-	20
SW-2A	-	14	-	16
SW-3A	-	12	-	16
SW-4A	-	14	-	18
SW-5A	-	12	-	16
SW-1B	-	16	-	20
SW-2B	-	14	-	18
SW-3B	-	12	-	16
SW-4B	-	14	-	16
SW-5B	-	12	-	16
Standard 50 µg/ml	18		24	
Control	-		-	

III. RESULTS AND DISCUSSION:

Chalcones were prepared in solvent ethanol via Claisen-Schmidt condensation between p-chloroacetophenone and substituted benzaldehyde. In this research work, we reduced the concentration of base to 2% for synthesizing the chalcones. The chalcones were then cyclised with guanidine nitrate to form corresponding pyrimidines, in order to reduce excess ethanol usage during filtration, a little more quantity of ethanol was used in reaction mixture so that the crystals formed were almost free from the red coloured impurity. The free $-NH_2$ group of substituted pyrimidine were acetylated and chloroacetylated with acetic anhydride and chloroacetyl chloride to get substituted pyrimidine derivatives (SW-1A - SW-5A and SW-1B - SW-5B).

The synthesized compounds were confirmed by IR, NMR spectral studies and screened for antimicrobial activity, the method used for the evaluation was cup-plate method. All the synthesized compounds shown significant amount of antimicrobial activity in 200 µg/ml concentrations. Among the synthesized compounds, SW-1A has got better antibacterial and antifungal activity. chloroacetylated compounds shown activity even in 100 µg/ml concentration against bacteria klebsiella pneumoniae. The standard trimethoprim and cotrimazole has shown better activity than synthesized compounds at 50 µg/ml concentration. Hence the synthesized compounds are not effective as standard reference drugs.

IV. CONCLUSION:

Pyrimidines are the important class of compounds due to their various pharmacological activities, the main objective of this research work was to synthesize, purify, characterize and evaluate antimicrobial activities of the substituted pyrimidine derivatives. The derivatives were tested for activities in 100 and 200 µg/ml concentrations. The synthesized compounds are not effective as standard reference drugs, but the substituted pyrimidine derivatives have notable antimicrobial activity and further research should be conducted on lead optimization to enhance the antimicrobial property.

REFERENCES:

[1]. Tripathi KD. Essentials of medical pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers: p. 667-71
[2]. Goodman & Gilman's: the pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill Book company.p. 1143-69
[3]. Furniss BS, Hannaford AJ, Roger V, Smith PWG, Tatchell AR. Vogel's textbook of practical organic chemistry. 4th ed. New

York: Longman Groups Limited; 1978: p.793-94

[4]. Ahluwalia VK, Parashar RK. Organic reaction mechanisms. 2nd ed. New Delhi: Narosa Publishing House; 2005: p.298-99

[5]. Sharma V, Chitranshi N, Agarwal AK. Significance and biological importance of pyrimidine in the microbial world. Int. J. Med. Chem. 2014; **2014**:1-35

[6]. El-Rayyes. A new route to the synthesis of substituted 2-pyrimidines. J. heterocycl. Chem. 1982; 19: 415-19

[7]. Palkar R B, Master H E. Synthesis of some new 6-amino-3-methoxyflavones. Ind. J. Chem. 2000; 39B:141-44

[8]. Banty AL. The antimicrobial susceptibility test; principle and practice, edited by Illus Lea and Febiger, (Philadelphia, USA), 180; 1976

[9]. Seely HW and Van Dermark PJ. Microbes in action: A laboratory manual of Microbiology, D.B.Taraporewala Sons and Co., Bombay, 1975;55-80