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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF 2, 4, 6-TRISUBSTITUTED PYRIMIDINE DERIVATIVES

Puspita Roy, Tanmoy Guria, Tanushree Singha, Benu Pani Sahu, Subbiah Ramasamy, Amalesh Samanta, & Tapan Kumar Maity*

Department of Pharmaceutical Technology, Synthetic and Natural Product Research Laboratory, Jadavpur University, Jadavpur, Kolkata-700032, West Bengal, India

*Corresponding author : Prof. Tapan Kumar Maity
e-mail : jutkmaity@yahoo.com, puspita.roy16@gmail.com, Mob.- 09432299486

ABSTRACT

In a wide search program toward new antimicrobial agents, we describe the synthesis of some pyrimidine derivatives (5a-d) from a series of chalcone analogues. These chalcone analogues were synthesized by condensation with substituted acetophenones and various aromatic aldehydes in ethanolic NaOH solution. The final compounds were characterized by using FT-IR and ^{1}HNMR spectroscopy. The antimicrobial activity of the novel products was evaluated against bacterial strains such as *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa* and *Bacillus pumilus*.

The result shows that drugs 5a and 5b have antimicrobial properties. Both the drugs were effective against five tested organisms among the listed organisms. Lowest Minimum Inhibitory Concentration of drug 5a and 5b was found at 200 µg/ml and the highest concentration was 400 µg/ml.

Keywords : Chalcone, Pyrimidine, MIC, Antimicrobial activity.

INTRODUCTION

At present, there is growing interest in the discovery of new antibacterial agents to bate against pathogenic microorganism, especially the bacteria resistant to the current antibiotics. Synthetic chemistry plays a key role in the development of new drug molecules for many years. With the advancement of modern chemistry and very sophisticated techniques it is has now become very easier to synthesize, characterize and evaluate new chemical entities (NCEs) [1].

Heterocycles form by far the largest of classical divisions of organic chemistry are of immense importance biologically and industrially [2].

Pyrimidines are 6-membered heterocyclic ring compounds composed of nitrogen and carbon. They are present throughout nature in various forms. Hundreds of pyrimidine-containing compounds have been found in biological system which control normal physiology. Pyrimidine ring is present in several pharmacologically active compounds, showing a wide range of biological activities, such as diuretic, anesthetic, anthelmintic, analgesic, anti-inflammatory etc [3, 4].

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against [5, 6].

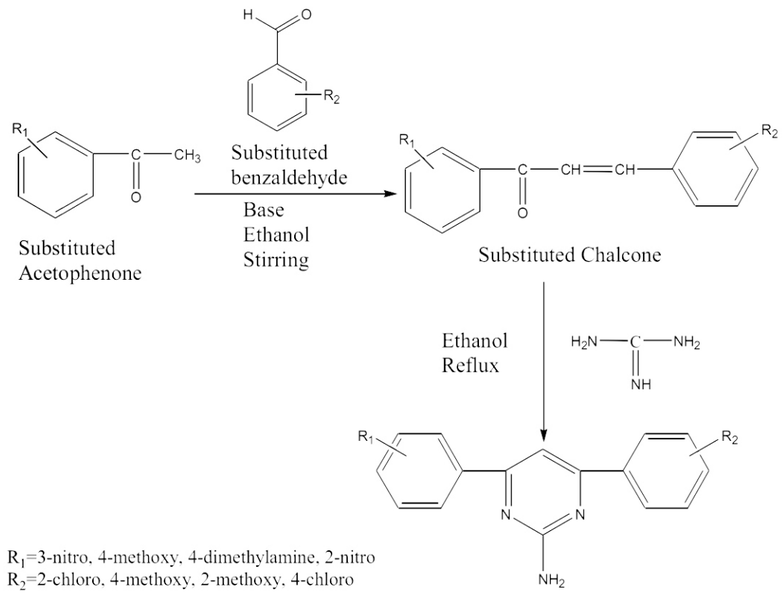
In an effort to develop potent anti-microbial agents, we have

reported the synthesis of 2, 4, 6-trisubstituted pyrimidine as well as their inhibitory activity against microbial germs.

MATERIALS AND METHODS

Scheme of Synthesis

Substituted acetophenone (0.01mol) (**1a-d**) and Substituted benzaldehyde (0.01mol) (**2a-d**) were dissolved in ethanol. The mixture was allowed to stir for several minutes at 5–10 °C. Then 20% ethanolic sodium hydroxide solution was slowly added dropwise to the reaction flask. The reaction solution was allowed to stir at room temperature for approximately 7-8 h. The reaction was completed by monitoring TLC. A precipitate was formed and then collected by suction filtration. Then the solid product was dried and recrystallized to get pure product (**3a-d**) [7].



2,4,6- trisubstituted pyrimidine

A mixture of (**3a-d**) (0.01 mol), guanidine hydrochloride 4 (0.01 mol) and 20% ethanolic sodium ethoxide solution were refluxed for 12-15 hours. After the completion of reaction, monitoring by TLC, the resultant mixture was cooled to room temperature and concentrated by rotary vacuum evaporator. The resulting solution was poured on crushed ice. The precipitated product 2, 4, 6 -tri substituted pyrimidine derivatives (**5a-d**) were filtered, washed with water, dried and recrystallized [8].

Evaluation of antimicrobial activity

The antimicrobial activity of the synthesized compounds were examined by *in vitro* test. There were several microorganisms both Gram-positive and Gram-negative which were used for this screening. They were thirteen in numbers and their names are as follows: 1. *Escherichia coli,* 2. *Salmonella typhi,* 3. *Staphylococcus aureus,* 4. *Vibrio cholera,* 5. *Streptococcus epidermidis,* 6. *Bacillus cereus* 7. *Bacillus subtilis* 8. *Micrococcus luteus* 9. *Salmonella typhimurium,* 10. *Psedomonas aeruginosa* 11. *Bacillus pumilus* 12. *Bacillus bronchisetica, and* 13. *Shigella dysenteria*. Amoxycillin, an antibacterial agent which is used widely as a clinical therapeutic agent was employed here as the reference material [9].

The media used for this study were primarily of two types:

1) Liquid culture media and 2) Solid culture media

1) Liquid culture media

Two types of liquid culture media were used, the composition of which are as follows :

Peptone Water

This medium was consisted of the following ingredients

Peptone————————1.0%

Sodium chloride—0.5%

Distilled water——100ml

pH adjusted————7.2-7.4

This medium was autoclaved at 15 lbs pressure for 15 minutes

Nutrient broth

This medium was consisted of the following ingredients

Peptone————————1.0%

Beef extract————1.0%

Sodium chloride——0.5%

Distilled water———100ml

pH adjusted————7.2-7.4

This medium was autoclaved at 15lbs pressure for 15 minutes

2) Solid culture media

a) Nutrient agar

This medium was consisted of the following ingredients

Peptone————————1.0%

Beef extract————0.5%

Sodium chloride——0.5%

Agar————————1.5%

Distilled water———100

These compositions were added one by one in 100 ml of distilled water and then it was melted for 30 min in water bath, then take it out and pH was adjusted to 7.2-7.4. Then this medium was autoclaved at 15 lbs pressure for 15 minutes and it was used for the cultivation of both gram positive and gram negative bacteria.

Nutrient agar for antimicrobial sensitivity test

This medium was consisted of the following ingredients

Peptone————————0.5%

Beef extract————0.5%

Sodium chloride——0.5%

Agar————————1.5%

Distilled water———100ml

pH ————————————7.2-7.4

Table 1 : MIC (µg/mL) Value of the Drug 5a

Organisms	Control	Drug 5a (µg/mL)						AM
		400	200	100	50	25	10	
B. cereus 11778	+	_	+	+	+	+	+	-
B. subtilis6633	+	_	+	+	+	+	+	-
M. luteus 10240	+	_	+	+	+	+	+	-
P. aeruginosa 25619	+	_	_	+	+	+	+	-
B. pumilus 14884	+	_	_	+	+	+	+	-

"+" represents no antimicrobial activity and "-" shows antimicrobial activity (AM - Amoxycillin). MIC of Amoxycillin against *B. cereus, B. subtilis, M. luteus, P. aeruginosa and B. pumilus* are 25, 25, 5, 100, 50 µg/mL respectively.

The drug was primarily tested for screening by using Agar Dilution [10]. Then the susceptible organisms were selected and Minimum Inhibitory Concentrations were determined as per NCCLS protocol [11] (NCCLS protocol, 1993) and by INT assay (*p* - iodo nitro teterazolium chloride). After that considering the best result against a particular organism was selected and effect on bacterial growth rate was observed.

Table 2 : MIC (µg/mL) Value of the Drug 5b

Organisms	Control	Drug 5b (µg/mL)						AM
		400	200	100	50	25	10	
B. cereus 11778	+	+	+	+	+	+	+	-
B. subtilis6633	+	_	+	+	+	+	+	-
M. luteus 10240	+	_	+	+	+	+	+	-
P. aeruginosa 25619	+	_	+	+	+	+	+	-
B. pumilus 14884	+	_	_	+	+	+	+	-

"+" represents no antimicrobial activity and "-" shows antimicrobial activity (AM - Amoxycillin). The microbial strains were the same as the previously mentioned.

Preparation of Inoculums

These strains were grown in Mueller-Hilton Agar (Merck India Ltd.) at 37 ^{0}C for 24 hrs and the suspension was prepared by matching a 0.5 McFarland standard [12].

Drug solution

The drug solution of synthetic drug was prepared by dissolving the crude drug in 1% DMSO (Dimethyl sulphoxide) solution along with sterile distilled water and makes the volume 2ml.

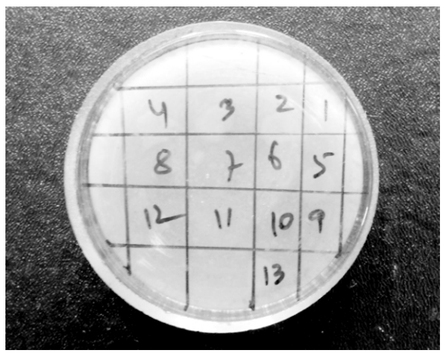


Figure 1 : Antimicrobial activity of 5a

Determination of MIC

The determination of MIC was done by agar disk diffusion method and confirmed by *p* - iodo nitrotetrazolium chloride (INT) assay. For disk diffusion 0.1 ml standard suspension of bacteria (2 x 10^6 CFU/ml) was spread over Muller-Hinton agar plates and disks containing drugs of different concentration were placed on every type microorganism containing plates. The inoculated plates were incubated at 37°C for 24 hrs and observed for zone of inhibition. The obtained MIC values were confirmed by INT assay in which two sets of nutrient broth were prepared to which 0.1 ml standard suspension of bacteria (2 x 10^6 CFU/ml) were added. To one set, specific amount of drug (10, 25, 50, 100, 200 and 400 µg/ml) were added and another was taken as control and finally *p* - iodo nitrotetrazolium chloride (INT) was added and incubated at 37°C for 24 hrs.

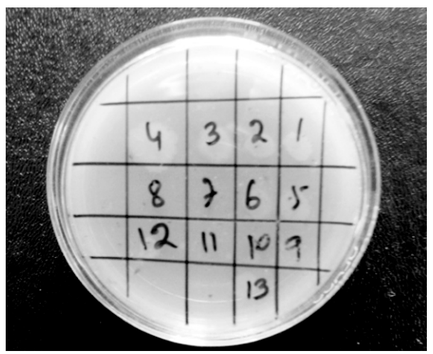


Figure 2 : Antimicrobial activity of 5b

Table 3 : MIC (µg/mL) Value of the Drug 5c

Organisms	Control	Drug 5c (µg/mL)						AM
		400	200	100	50	25	10	
B. cereus 11778	+	+	+	+	+	+	+	-
B. subtilis6633	+	+	+	+	+	+	+	-
M. luteus 10240	+	+	+	+	+	+	+	-
P. aeruginosa 25619	+	+	+	+	+	+	+	-
B. pumilus 14884	+	+	+	+	+	+	+	-

"+" represents no antimicrobial activity and "-" shows antimicrobial activity (AM - Amoxycillin).

Table 4 : MIC (µg/mL) Value of the Drug 5d

Organisms	Control	Drug 5d (µg/mL)						AM
		400	200	100	50	25	10	
B. cereus 11778	+	+	+	+	+	+	+	-
B. subtilis6633	+	+	+	+	+	+	+	-
M. luteus 10240	+	+	+	+	+	+	+	-
P. aeruginosa 25619	+	+	+	+	+	+	+	-
B. pumilus 14884	+	+	+	+	+	+	+	-

"+" represents no antimicrobial activity and "-" shows antimicrobial activity (AM - Amoxycillin).

RESULT & DISCUSSION

In the present study, four pyrimidines derivatives were synthesized from chalcone derivatives. Specifically 2, 4, 6-trisubstituted pyrimidines were synthesized from the chalcones obtained from substituted acetophenones and substituted benzaldehydes. The synthesized molecules were purified through filtration and recrystallization techniques. The molecular structures of the synthesized compounds were defined using IR and ^{1}H NMR spectroscopic methods. Antimicrobial activity was evaluated using different bacterial strain such as *B. cereus*, *B. subtilis*, *M. luteus*, *P. aeruginosa*, and *B. pumilus.*

The drug 5a and 5b showed antimicrobial properties. The drugs 5a and 5b were effective against five tested organisms among the listed organisms. Lowest MIC of drug 5a and 5b was found at concentration 200 µg/ml and the highest concentration was 400 µg/ml. The drug 5c and 5d have no antimicrobial activity.

From the results against the microorganisms of the test samples we can conclude that the drug is broad spectrum i.e. effective on both gram positive and gram negative organisms, which is more effective on enteric organisms. Moreover, by analyzing bacterial growth curve we can conclude that the synthetic drug inhibits growth of tested bacterial strains at MIC and exert its effect as a bacteriostatic agent.

Chemical data of synthesized compounds

4-(2-chlorophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine (5a): MF: $C_{15}H_{11}N_4O_2Cl$; MW: 352.5g/mol; % yield: 52%; MP: 175-178°C; IR (V_{max} cm^{-1}): 3371 (NH), 3000 (ArC-H), 1617 (C=N), 1570 (C=C), 754 (ArC-Cl), 1470 (ArC-NO_2); ^{1}H NMR: δ 5.262 (s, 2H, NH_2), δ 6.62-7.47 (m, 8H, Ar-H), δ 7.86 (s, 1H, C5-H).

4, 6-bis (4-methoxyphenyl) pyrimidin-2-amine (5b): MF: $C_{18}H_{17}N_3O_2$; MW: 307g/mol; % yield:55%; MP: 163-168°C; IR (V_{max} cm^{-1}): 3370 (NH), 3000 (ArC-H), (C=N), 1563 (C=C), 1174, 1239 (ArC-OCH_3); ^{1}H NMR: δ 5.188 (s, 2H, NH_2), δ 6.98-7.36 (m, 8H, Ar-H), δ 8.01 (s, 1H, C5-H), 3.87 (d, 6H, OCH_3).

4-[4-(dimethyamino) phenyl]-6-(2-methoxyphenyl) pyrimidin-2-amine (5c): MF: $C_{19}H_{20}N_4O$; MW: 320g/mol; % yield: 51%; MP: 215-218°C; IR (V_{max} cm^{-1}): 3300(Ar-NH), 1060,1126,1243(OCH_3), 1608,1673(C=N), 1570(C=C), 1525(CH=CH), 1357(NH_2); ^{1}H NMR: δ 5.05 (s, 2H, NH_2), δ 6.48-7.43 (m, 8H, Ar-H), δ 7.95 (s, 1H, C5-H), δ 3.88 (d, 3H, OCH_3), 2.79 (s, 6H, -$N(CH_3)_2$).

4-(4-chlorophenyl)-6-(2-nitrophenyl) pyrimidin-2-amine (5d): MF: $C_{15}H_{11}N_4O_2Cl$; MW: 352.5g/mol; % yield:78%; MP:179-185°C; IR (V_{max} cm^{-1}): 3366 (NH), 3090 (ArC-H), (C=N), 1568 (C=C), 710, 790 (ArC-Cl), 1492 (ArC-NO_2); ^{1}H NMR: δ 5.202 (s, 2H, NH_2), δ 6.80-7.46 (m, 8H, Ar-H), δ 7.93 (s, 1H, C5-H).

CONCLUSION

The overall results of the present work reflect a preliminary stage in developing of pyrimidine derivatives in targeting microbial cells. The probable mechanism of action against the microorganisms of the drug can be characterized by further studies. Moreover, to bring these molecules from laboratory to patients more scientific study should be done.

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF SOME NOVEL PYRIDAZINE DERIVATIVES

Shadab A. Siddiqui[1*], Anees A. Siddiqui[2], K. Nagarajan[1]

[1]KIET School of Pharmacy, Ghaziabad, 201206

[2]Department of Pharm. Chem. F/O Pharmacy, Jamia Hamdard, New Delhi. 110062

*Author of Correspondence : Shadab A. Siddiqui, Asst. Prof., KIET School of Pharmacy, Ghaziabad, U.P. 201206

Mob. +91-9999967128, e-mail: shadabhamdard@gmail.com

ABSTRACT

A series of 2-substituted phenyl-10H-Pyridazine (6, 1-b) quinazoline-10-one (**d-i-vii**) were synthesized. These compounds were evaluated for their anticonvulsant activity. Compound **d-iii**, and **d-v** showed 100% protection. Compound **d-i**, **d-iv**, and **d-vi** showed 66.67% protection and compound **d-vii** showed 83.33% protection. Anticonvulsant activity of these compounds was compared with phenytoin.

Key words: 2-substituted pyridazine, anticonvulsant, pyridazinones.

INTRODUCTION

The pyridazine ring system is a 1,2-diazine or o-diabenzene. The name pyridazine was suggested by Knorr[1], however, the first substituted pyridazines were prepared in 1886 by Fischer[2], and pyridazine itself was prepared by Tauber[3] in 1895. During last two decades numbers of pyridazine/ pyridazinones have been synthesized due to their effect on cardiovascular system pyridazine/pyridazinone derivatives were reported to exhibit lots of pharmacological activities such as anti-inflammatory[4], antibacterial[5], antithrombotic[6], antihypertensive[7], hypotensive[8], cardiotonic[9], diuretic[10] and anti-HIV[11] activities. A lot of work have been done on pyridazine/pyridazinones in the last few years for their various pharmacological activities and anticonvulsant activity[16] also. In continuation to the work on pyridazine/pyridazinone ring system, we have synthesized some new pyridazine derivatives and evaluated them for anticonvulsant activity by non-invasive methods.

EXPERIMENTAL SECTION

(i) Synthesis of α-benzoyl propionic acid (a): After suspending anhydrous aluminium chloride (15 gm.) in dry benzene (50 ml) under anhydrous conditions, the contents were refluxed on a water bath. Succinic anhydride (10 gm) then added in small proportion through the condenser (care should be taken that after adding each lot, the second lot was added only when first lot reacted completely). After complete addition, the reaction mixture was refluxed for 6 hours. A solution of conc. Hydrochloric acid (2.5ml) in ice –cold water (7.5ml) was then added to the reaction mixture and the contents concentrated to a small volume by heating on a water bath. On cooling a crystalline compound separated out which was filtered and re-crystallized from water to give a colorless compound.

(ii) Synthesis of 6-phenyl 2,3,4,5-tetrahydropyridazine-3-one (b): To a solution of α-benzoyl propionic acid in methanol (30 ml) was added hydrazine hydrate(1ml) and sodium acetate (0.5 gm) and the contents were refluxed for 6 hours. After completion of the reaction, methanol was distilled off and residue poured into cold water. The solid which separated out, was filtered and re-crystallized from methanol.

(iii) Synthesis of 3-chloro-6-phenylpyridazine (c): To a solution of 6-phenyl 2,3,4,5-tetrahydropyridazine-3-