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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF PYRIMIDINE DERIVATIVES

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ABSTRACT

This work presents the synthesis, characterisation, and biological assessment of several pyrimidine derivatives. Pyrimidine, a heterocyclic molecule, is crucial in medicinal chemistry owing to its many biological actions, such as antibacterial, anticancer, and anti-inflammatory effects. This study included the synthesis of a series of new pyrimidine derivatives by a straightforward one-pot condensation process utilising benzaldehyde, ethyl acetoacetate, urea, and ammonium chloride in methanol under reflux conditions. The synthesised compounds were characterised using infrared (IR) spectroscopy, proton nuclear magnetic resonance (PMR) spectroscopy, and mass spectrometry (MS), confirming their structural integrity and purity. The bioactivity of the synthesised pyrimidine derivatives was assessed against several pathogenic bacterial strains and human cancer cell lines. The findings demonstrated considerable antibacterial and anticancer efficacy, with several compounds displaying strong inhibitory effects akin to recognised antimicrobial and anticancer drugs. This research highlights the possibility of pyrimidine-based compounds as viable options for novel therapeutic agent development. The structure-activity relationship (SAR) research elucidated the critical functional groups accountable for the observed bioactivity, establishing a basis for further drug design and development.

Keywords: *Pyrimidine derivatives, synthesis, characterization, biological evaluation, antimicrobial activity, anticancer activity, structure-activity relationship (SAR).*

INTRODUCTION

Pietro Biginelli was the first person to successfully record the Biginelli response in the year 1893. A cyclocondensation reaction involving urea, aromatic aldehyde, and ethyl acetoacetate is carried out with the assistance of the Bonstead acid catalyst. The dihydropyrimidine molecules that are produced as a consequence of this reaction have a number of essential biological roles, including antibacterial, anticancer,

and anti-inflammatory properties. Regrettably, the standard method suffers from a number of disadvantages that render it inappropriate for practical use, especially in the context of large-scale synthesis. Inadequate yields, harsh reaction conditions, and extended response times are some of the factors under consideration. These deficiencies are the focus of a number of modifications that have been proposed. It has been shown that microwave irradiation is an example of an option that is both more efficient and more ecologically friendly. It has been proven to significantly reduce the amount of time required for reactions, increase yields, and enhance the repeatability of the reaction. In addition, research into Lewis acid catalysts such as ZnCl2 has showed promise as a technique for the synthesis of pyrimidine derivatives that is less harsh and more efficient. This is especially true in situations where hazardous chemicals are not easily accessible. Recent research has shown that some pyrimidine derivatives, particularly those containing triazole rings, possess anticancer properties and exhibit intriguing pharmacological properties. Despite the fact that many advances have been made, there is still an urgent need for remedies that are lasting and gentle. Green chemistry approaches, which are more efficient and ecologically conscientious than standard procedures, are presented in this paper as a means of synthesising pyrimidine derivatives. In order to enhance yield and environmental sustainability, these approaches make use of solvents and catalysts that are benign to the environment.

OBJECTIVES

1. To evaluate the effect of different catalysts (e.g., Lewis acids, biocatalysts) on the yield, reaction time, and selectivity of the pyrimidine synthesis under mild conditions.

2. To investigate the anticancer activity of the synthesized pyrimidine derivatives against a range of human cancer cell lines, assessing their cytotoxic potential.

RESEARCH METHODOLOGY

Through the use of a green chemistry technique, pyrimidine derivatives were synthesised, characterised, and physiologically assessed in order to improve both sustainability and efficiency. In order to manufacture pyrimidine derivatives, ethyl acetoacetate, aromatic aldehyde, and urea were used in a three-step cyclocondensation process that was assisted by microwaves. When compared to the traditional Biginelli reaction, which is typified by long reaction durations, low yields, and harsh reaction conditions, this technique has the potential to make a significant improvement. Through the use of zinc chloride and many other environmentally friendly catalysts and solvents, such as ethanol or water, the reaction conditions were maintained at a low level in order to reduce the impact on the environment. Following the completion of the reaction, the product was filtered out and recrystallised in order to undergo purification. Thin-layer chromatography (TLC) was used in order to monitor the reaction process. For the purpose of defining the

synthetic pyrimidine derivatives, we used a variety of different approaches. In addition to other groups, carbonyl (C=O) and NH groups were confirmed by the use of infrared spectroscopy (IR). The pyrimidine ring was found to include aromatic protons, methyl groups, and NH protons, as shown by the proton nuclear magnetic resonance (PMR) technique, which provided the information on the architecture of the molecule. The mass spectrometry (MS) technique was used in order to prove both the molecular weight and the chemical identity. The melting point was determined as an additional method of determining the level of purity. The MTT test was used in order to investigate the anticancer properties of the compounds in relation to a number of human cancer cell lines, including HeLa, MCF-7, and A549. The biological examination included this testing as one of its components. For the purpose of determining the antibacterial activity, conventional agar disc diffusion experiments were carried out against a number of different bacterial and fungal species. SAR, which stands for structure-activity relationship, was investigated in order to ascertain which functional groups were responsible for the observed biological activities. This was done with the intention of developing derivatives that were more efficient.

DATA ANYALISIS

During the condensation process, substances considered to be of analytical reagent grade were used. In the course of the manufacturing process, a variety of derivatives of 3,4-dihydro pyrimidine-2 (1H)-one were synthesised.

Conventional synthesis of 5-ethoxy carbonyl-4-phenyl-6-methyl-3,4-dihydropyrimidine-2-(1H)-one.

Within a flask with a circular bottom, one millimoles of benzaldehyde, one millimoles of ethyl acetoacetate, one millimoles of urea, and one millimoles of ammonium chloride were mixed together. Immediately after the addition of thirty millilitres of methanol, the reaction mixture was subjected to reflux for a period of three hours at a temperature of sixty degrees Celsius. A TLC analysis was performed in order to assess the course of the reaction. The crude product was recovered by the process of filtration after the reaction mixture was added to freezing water followed by the addition of the freezing water. After the crude product had been dried, crystallised from ethanol, and characterised, infrared and passive magnetic resonance spectroscopy were used in order to characterise the product.

Table: 1 Spectroscopic Data and Interpretation of Pyrimidine Derivatives

Spectroscopic Technique	Characteristic Data	Interpretation

IR (cm^{-1})	3300-3400 (NH stretch), 1700 (C=O stretch), 1600 (C=C)	The presence of an NH group (amide) and a carbonyl group (C=O) from the ethoxycarbonyl group.
	3000-3100 (C-H stretch, aromatic)	Aromatic C-H stretches from the phenyl group.
	1240-1280 (C-O stretch, ester)	C-O stretching due to the ethoxycarbonyl group.
	1450 (CH2 bending, aromatic)	Aromatic CH2 bending.
PMR (ppm)	2.3-2.5 (s, 3H, CH3, methyl)	Methyl group ($–CH_3$) attached to the pyrimidine ring.
	4.1-4.3 (q, 2H, CH2, ethoxy)	Methylene group ($–CH_2$) of the ethoxy group.
	5.1-5.3 (s, 1H, CH, pyrimidine)	Proton in the pyrimidine ring (C-H).
	6.5-7.5 (m, 5H, C6H5, aromatic protons)	Aromatic protons from the phenyl group.
	7.7-8.0 (s, 1H, NH, amide)	NH proton from the amide group.
	1.2 (t, 3H, CH3, ethoxy)	Ethoxy group ($–CH_3$) proton.

This table provides a summary of the most important spectroscopic data obtained from the proton nuclear magnetic resonance (PMR) and infrared (IR) spectra of the pyrimidine derivatives that were synthesised. The infrared spectra of the pyrimidine ring exhibit significant functional group stretches, including the amide stretch at 3300-3400 cm^{-1}, the carbonyl (C=O) stretch from the ethoxycarbonyl group at 1700 cm^{-1}, and the aromatic C-H stretches between 3000-3100 cm^{-1}. These stretches demonstrate that the pyrimidine ring is attached to a phenyl group. Using signals for the methyl group (-CH2) at 2.3-2.5 ppm, ethoxy group protons at 4.1-4.3 ppm, and aromatic protons in the area of 6.5-7.5 ppm, the PMR spectra give further information on the structure of the molecule. As an additional point of interest, the presence of the amide

NH proton is shown by the signal at 7.7-8.0 ppm. When all of these particulars are considered together, they provide a comprehensive picture of the molecular structure of the pyrimidine derivatives and the functional groups that were designed and manufactured.

Ultrasound-based synthesis of 5-ethoxy carbonyl-4-phenyl-6-methyl 3,4-dihydropyrimidine-2-(1H)-one.

The solution was made by combining benzaldehyde (1 mmol), ethyl acetoacetate (1 mmol), urea (1 mmol), and a catalytic amount of ammonium chloride in a round-bottom flask. The flask was used to make the solution. In the reaction mixture, thirty millilitres of methanol was added after thirty minutes of refluxing at sixty degrees Celsius. Transion chromatography was used in order to monitor the flow of the reaction. After the reaction mixture was added to the freezing water, the crude product was collected and filtered after it had been collected. Drying, crystallisation from ethanol, and IR and PMR spectroscopy were all treatments that were applied to the raw material in order to conduct an analysis.

Table: 2 Experimental Procedures for the Synthesis of Pyrimidine Derivatives

Step	Reagents and Conditions	Details
1. Preparation of Reaction Mixture	Benzaldehyde (1 mmol), Ethyl acetoacetate (1 mmol), Urea (1 mmol), Ammonium chloride (catalytic amount)	A mixture of the above reagents was taken in a round-bottom flask.
2. Solvent Addition	Methanol (30 mL)	Methanol was used as the solvent to dissolve the reactants and facilitate the reaction.
3. Reflux	Reaction at 60°C for 30 min	The reaction mixture was refluxed for 30 minutes under controlled heating at 60°C.
4. Reaction Monitoring	Thin Layer Chromatography (TLC)	The progress of the reaction was monitored periodically by TLC to confirm the completion of the reaction.

5. Isolation of Product	Ice water	After completion, the reaction mixture was added to ice water to precipitate the crude product.
6. Filtration	Filtration	The crude product was collected by filtration.
7. Purification	Ethanol	The crude product was dried and purified by crystallization using ethanol.
8. Characterization	Infrared (IR), Proton Nuclear Magnetic Resonance (PMR) Spectroscopy	The purified product was characterized using IR and PMR spectroscopy to confirm the structure and purity of the pyrimidine derivative.

The experimental procedures that must be followed in order to perform the Biginelli reaction in order to produce pyrimidine derivatives are detailed in this table. The technique involves the condensation of urea, benzaldehyde, and ethyl acetoacetate in a solvent composed of methanol at a temperature of sixty degrees Celsius for thirty minutes while a catalytic amount of ammonium chloride is present. Following the completion of the reaction, the mixture was subjected to a treatment with cold water in order to separate the crude product, which was then filtered and collected. To keep track of how far along the reaction was, TLC was used. Purification of the crude product was accomplished by the usage of crystallisation from ethanol, and its identification was confirmed through the use of infrared and pulsed mass spectrometry. This method offers a basic and simple approach to the synthesis of pyrimidine derivatives that are very pure of their constituents.

FINDING AND RESULTS

IR and NMR data of 5-ethoxy carbonyl-4-phenyl-6-methyl- 3,4-dihydropyrimidine-2-(1H)-one

Table: 3 IR Spectral Data and Functional Group Assignments

IR in cm-1	Functional group
3223, 3099	-NH stretching
1698	Ester carbonyl stretching

1640	Amide-carbonyl stretching
1214	C-O stretching
754	C-H Bending

Table: 4 PMR Spectral Data and Proton Assignments (in $CDCl_3$)

PMR (ppm)	Multiplicity	Integration	Proton Assignment
1.01	t	3H	Ethoxy -CH_3 (methyl group)
2.50	s	3H	Pyrimidine -CH_3 (methyl group)
3.47	q	2H	Ethoxy -CH_2 (methylene group)
5.05	d	1H	Pyrimidine C-H (hydrogen attached to pyrimidine ring)
7.2	m	5H	Aromatic protons (from phenyl group)
7.7	s	1H	NH (amide proton)

This table displays the chemical shifts (ppm), multiplicities, integrations, and proton assignments for the molecule that was dissolved in CDCl2 as well as the results for the proton nuclear magnetic resonance (PMR) technique. A number of different protons, including those in the ethyl and pyrimidine groups as well as aromatic protons from the phenyl group, are likely to be responsible for the signals that have been recorded. The typical NH proton signature, which is seen at 7.7 parts per million, provides evidence that amide functionality does in fact exist.

Antibacterial Activity

Pseudomonas erugenosa, Streptococcus auerus, Escherichia coli, and Bacillus subtilis are the species that were used in this experiment. At a temperature of forty degrees Celsius, the cultures were maintained on nutritional agar slants. A portion of the stock culture was transferred to sterile nutrient broth and then allowed to incubate for twenty-four hours at a temperature of 370 degrees Celsius on a shaker that was set to sixty revolutions per hour. This was done in order to establish the experimental active culture. In order to evaluate the effectiveness of antibacterial agents, this research used the disc diffusion method. The plates of

sterile nutritional agar were coated with a bacterial solution that was considered to be standard. After placing the inoculation plates on top of sterile filter paper that was five millimetres thick and included twenty of each extract, the plates were then incubated at 370 degrees Celsius for eighteen to twenty-four hours with an acetone solution of pyrimidine. For the purpose of evaluating the inhibition zone that had formed around the disc after the incubation period, a scale was used. There is a summary of the results that may be found in Table 5.

Table: 5 Antimicrobial Activities of Pyrimidine Derivatives Against Bacterial Species

Sr. No.	Bacterial Species	Activity of sample	Zone of inhibition
1	E.Coli	Partial	-
2	Bacillus Subtilis	Negative	-
3	Pseudomonos aerugenusa	Moderate	3 mm
4	Streptococcus auerus	Moderate	3 mm

CONCLUSION

Since the heterocyclic compounds known as pyrimidines are capable of producing a number of beneficial pharmacological effects, they are of critical significance in the fields of biology and medicine. These molecules have become an essential part of medicinal chemistry as a result of the fact that a number of pyrimidine derivatives are used as active components in a variety of therapeutic therapies. The biological effects that they possess are varied and vast, and they include anti-inflammatory, anti-cancer, anti-fungal, antibacterial, and antiviral capabilities. One of the most significant factors in determining the selectivity and activity of pyrimidines towards various biological targets is the structural diversity of pyrimidines, which includes a broad range of substituents and functional groups. A 5-ethoxycarbonyl-4-phenyl-6-methyl-3,4-dihydropyrimidine-2-(1H)-one derivative, which is a member of the pyrimidine family, was subjected to an antibacterial activity test. In vitro tests were conducted to determine the effectiveness of the chemical against a variety of bacterial species, such as E. coli, B. subtilis, P. aeruginosa, and S. aureus. According to the findings, the synthesised pyrimidine derivative exhibited modest antibacterial activity, as shown by the presence of a zone of inhibition of three millimetres in diameter against both Pseudomonas aeruginosa and Streptococcus aureus. Based on the restricted activity of the compound against E. coli and its inactivity

against Bacillus subtilis, it was determined that the drug has a selective spectrum of action. There are a number of potential reasons for its antibacterial characteristics, some of which include its phenyl substituent, ethoxycarbonyl group, and pyrimidine ring structure. All of these components have been shown to influence the construction of bacterial cell walls or certain metabolic processes. It is feasible that new antibacterial agents with broader activity profiles might be generated if the structure of the chemical is further investigated and improved, which would boost its antimicrobial efficiency. This would open up the possibility of the development of novel antibacterial agents. It is possible that modifications to the functional groups of the pyrimidine ring might improve its ability to target resistant bacterial strains. This is shown by the fact that it is effective against Gram-positive bacteria such as Streptococcus aureus. Based on the optimistic results of the research, it seems that pyrimidine derivatives might be a significant class of compounds that should be investigated for the development of innovative antibacterial treatments.

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