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Synthesis, Characterization, And Evaluation Of Novel Heterofused Pyrimidine Derivatives As Potential Therapeutic Agents

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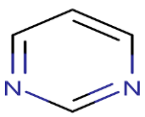
ABSTRACT: Due to their broad range of biological activities, heterofursed pyrimidine derivatives have been receiving a lot of attention, especially in the context of antimicrobial and anticancer treatments. The goal of this work was to investigate the pharmacological potential of newly discovered heterofused pyrimidine derivatives by synthesizing them using conventional methods. a combination of IR, NMR, and mass spectrometry to evaluate the synthesized compounds structurally, it emerged whether the target derivatives were effectively synthesized. By using human cancer cell lines in vitro cytotoxicity assessments have been used to evaluate their anticancer potential. The results demonstrated strong activity with IC_{50} values equivalent to those of conventional chemotherapeutic drugs.
Additionally, antimicrobial activity was evaluated against a panel of fungal and bacterial strains; derivatives containing sulfur were found to have particularly promising broad-spectrum efficacy. These results imply that the produced heterofused pyrimidine derivatives may be useful as starting points for the synthesis of novel antibiotics and anticancer drugs. Future efforts in drug discovery should focus on further optimizing their chemical structures and researching their mechanisms of action.

KEYWORDS: Drug discovery, pharmacological evaluation, broad-spectrum efficiency, structural characterization, derivatives containing sulfur, cytotoxicity tests, derivatives of heterofurused pyrimidines, antibacterial and anticancer activities.

INTRODUCTION:

Pyrimidine derivatives were a significant group of heterocyclic compounds used in medical chemistry due to their biological properties. These substances possess antimicrobial, antiviral, anticancer, and anti-inflammatory features, among many other biological activities.
A fundamental component of nucleic acids, pyrimidines is the structural components of RNA as well as DNA. In addition, the enhanced toxicological profiles and their fused analogs offer being fusing with other heterocyclic systems received a lot of emphasis. Constructing heterofused pyrimidine derivatives—especially those with additional atoms of nitrogen, oxygen, or sulfur—has grown to be an important area of research in the research for fresh medicinal substances.[1]
Heterofused pyrimidines have generated attention to searches for innovative psychologically active substances because of their structural diversity as well as ability to interact with biological targets.[2] A number of compounds demonstrate considerable anticancer activity, highlighting their promise as cancer therapeutics. Due these substances may disrupt DNA synthesis and inhibit important enzymes involved in cell proliferation, these frequently demonstrate increased potency and selectivity against cancer cell lines. This happens because they include heterofused pyrimidine moieties.[3]
To broaden their therapeutic Distance, these compounds are also known to have antibacterial activity against a number of bacterial and fungal species.[4]
To be able to yield heterofused pyrimidines, functionalized pyrimidine rings are usually incorporated into other heterocyclic systems using cyclization processes. Modifications to a structure that may enhance biological activity are feasible via this approach.[5] Recent improvements in synthetic methods have rendered it simpler to successfully assemble these intricate molecules, offering up new avenues for studying their biological properties. Traditional synthetic techniques, like thermal and solution-phase processes, are still commonly used because they can generate high-purity compounds that can be analyzed biologically.[6]
The objective for this project was to develop an array of distinctive heterofused pyrimidine derivatives and examine their potential biological properties as antibacterial and anticancer medicines. To verify the effective formation of the target compounds, structural characterization has been carried out using mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy. In vitro cytotoxicity studies against human cancer cell lines and antimicrobial testing against a panel of bacterial and fungal pathogens are the primary objectives of this biological evaluation. Studying these compounds' structure-activity connections (SAR) and identifying potential lead candidates to further treatment development was the objective.



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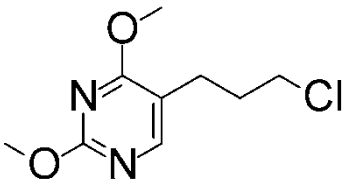
The urgent need for new drugs for the treatment of drug-resistant diseases and cancer is the motivation driving this research. The development of chemoresistance is still a key concern in cancer treatment, despite excellent advances. Therefore, novel compounds with distinctive ways of action are constantly searched sought. Furthermore, there is a growing need for innovative antibiotics with broad-spectrum efficacy due to the increasing prevalence of antimicrobial resistance. Heterofused pyrimidine derivatives offer an effective strategy to tackling these global health problems.[7]

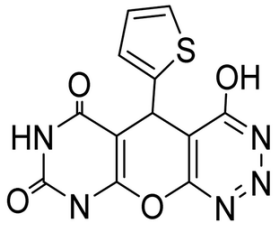
OBJECTIVE: The aim of the present investigation is to synthesize a number of heterofused pyrimidine derivatives, evaluate their biological activity having a focus on antimicrobial and chemotherapeutic abilities, and evaluate their structural properties using a variety of spectroscopic techniques. The goal of this research is to optimize these derivatives for the creation of medications through inquiry at their structure-activity relationships (SAR).

SCOPE: The primary goals of the study will be to synthesis heterofused pyrimidine derivatives, describe their structures, and evaluate their potential as cancer and bacterial infection therapeutic agents.

LITERATURE REVIEW:
In medicinal chemistry, pyrimidine and its derivatives have attracted an enormous amount of attention due to their numerous biological properties. Pyrimidines' heterocyclic structure exhibits enhanced medicinal properties, especially when participated in with other rings, making them intriguing possibilities for an array of medicinal purposes.
Zhu et al. (2017) reported that the compounds in vitro effectiveness was improved via modifications to the structure of the pyrimidine scaffold, such as alkylation or the incorporation of heteroatoms at various locations. Discovering pyrimidine-based drugs, especially 5-fluorouracil and its analogues, has created new avenues for the advancement of cancer treatments. Compounds fused with oxygen- or sulfur-containing rings show selective cytotoxicity towards cancer cells, sparing healthy cells, whereas heterofused pyrimidines have shown greater cytotoxicity against an array of cancer cell lines. Additionally, by increasing the compounds' binding affinity to bacterial and fungal enzymes, the addition of heteroatoms like oxygen and sulfur to the fused ring framework significantly enhanced the antimicrobial profile.[8]



As reported by Roy et al. (2018), fused pyrimidines containing heteroatoms of oxygen and sulfur exhibited enhanced antimicrobial activity. The study emphasized that novel environmentally friendly methods for creating heterofused pyrimidines, like solvent-free reactions and microwave-assisted synthesis, have been made possible by recent developments in green chemistry. These techniques enable the quick and environmentally friendly synthesis of heterocycles based on pyrimidines.[9]



Wang et al. (2018) demonstrated that since pyrimidine derivatives possess a more powerful binding affinity to enzymes such bacterial DNA gyrase and dihydrofolate reductase, proteins exhibit enhanced antimicrobial effects if fused with triazole or oxazole rings. In addition, research showed that particular heterofused pyrimidines may successfully halt the growth of colon and breast cancer cells by causing ROS-mediated cell death, DNA intercalation, and enzyme inhibition. Furthermore, it was shown that introducing bulky alkyl or aryl groups to the pyrimidine ring at position between four and five enhances its antibacterial and anticancer properties by promoting cellular absorption and lowering metabolic breakdown.[10]

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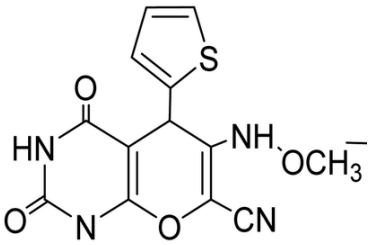
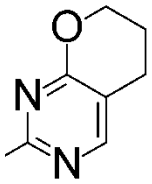
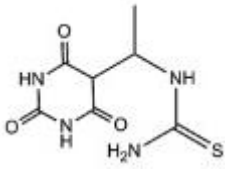


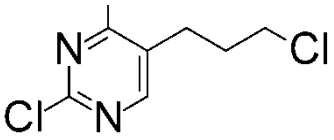
According to Balbi et al. (2019), the presence of heterocyclic moieties enhanced the pyrimidine derivatives' penetration into bacterial cell membranes through decreasing their lipophilicity, which is essential for their antibacterial activity. Notable antifungal activity against species like Candida albicans was also demonstrated by the study.[11]



Kumar et al. (2019) reported that nitrogen-containing fused pyrimidines shown significant antibacterial action against Staphylococcus aureus and Escherichia coli, among other Gram-positive and Gram-negative bacteria. Furthermore, it has been discovered that electron-withdrawing groups on the pyrimidine ring, like halogens, increase the efficacy of chemotherapy through promoting interactions with target enzymes.[12]

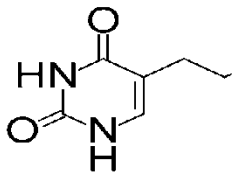


A variety to pyrimidine-based compounds have been identified by Gupta et al. (2020) and have proceeded to preclinical studies due to their promising in vivo and in vitro anticancer activity. These findings open the door to more research into the therapeutic uses of heterofused pyrimidines and propose that they may be useful in the development of new drugs, especially for the treatment of cancer and infectious disorders.[13]



The development of heterofused pyrimidine derivatives with potential anticancer properties is the primary objective of Patel et al. (2021). The study they conducted yielded encouraging results, suggesting that mitochondrial-mediated apoptosis selectively inhibits colorectal cancer cells. This work demonstrated the utility of heterofused pyrimidines in the development of tailored cancer therapeutics by improving cellular absorption by altering the pyrimidine scaffold structurally.[14]

The antibacterial property of fused pyrimidine compounds with heteroatoms of sulfur and nitrogen was investigated by Singh et al. (2022). Pseudomonas aeruginosa and Staphylococcus epidermidis were among the multidrug-resistant bacterial strains that the study successfully blocked from growing, demonstrating the compounds' potential as novel antimicrobial agents to combat resistant diseases.[15]



Zhang et al. (2023) study examined the application of pyrimidine-based substances in the creation of antiviral medications, with a particular emphasis on the suppression of viral replication in RNA viruses like SARS-CoV-2.

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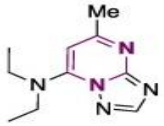
According to the study, certain heterofused pyrimidines that target viral proteases shown robust antiviral action in vitro, making them viable candidates for additional clinical testing against new viral threats.[16]



MATERIAL AND METHODS:

Chemicals and Reagents:

- Urea
- Ethyl acetoacetate
- Appropriate solvents like ethanol, methanol and dimethylformamide (DMF)
- Substituted benzylaldehyde (as starting material)
- Purification material such as activated charcoal and silica gel.
- Various catalysts like piperidine or KOH for reactions.

Methods:

1. **Synthesis of Ethyl 6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives (1c-1d):**

- Mix urea, phosphorus pentoxide, benzaldehyde, and ethyl acetoacetate.
- After a 3-minute reflux, cool down and move to crushed ice.
- From ethanol, filter, dry, and recrystallize.
- Monitor the reaction using TLC.

2. **Synthesis of Ethyl 6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-hydrazinecarbothioamide derivatives (2c-2d):**

- React ethyl 6-methyl-2-oxo/thioxo-4-substituted-tetrahydropyrimidine-5-carboxylate with thiosemicarbazide in glacial acetic acid.
- Microwave for 5 minutes.
- Cool, filter, dry, and recrystallize from ethanol.

3. **Synthesis of 5-(5-amino-1,3,4-thiadiazole-2-yl)-6-methyl-4-substituted-3,4-dihydropyrimidine-2(1H)-one/thione derivatives (3c-3d):**

- Dissolve hydrazinecarbothioamide in concentrated H_2SO_4.
- Stir for 1 hour, cool, and pour into crushed ice.
- Recrystallize from ethanol.

4. **Synthesis of 5-(5-(benzylideneamino)-1,3,4-thiadiazole-2-yl)-6-methyl-4-substituted-3,4-dihydropyrimidine-2(1H)-one/thione derivatives (4c-4d):**

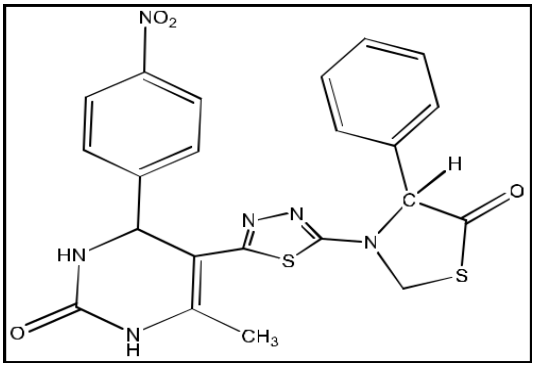
- React compound (3a-p) with benzaldehyde, sodium acetate, and H_2SO_4 in ethanol under microwave for 3 minutes.
- Remove solvent, filter, and recrystallize.

5. **Synthesis of 6-methyl-5-[5-(5-oxo-4-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]-4-substituted-3,4-dihydropyrimidin-2(1H)-one/thione derivatives (5c-5d):**

- Microwave compound (4a-p) with mercaptoacetic acid and $ZnCl_2$ in DMF.
- Wash with $NaHCO_3$ solution, dry, and recrystallize.

STRUCTURE AND IUPAC NAME OF SYNTHESIZED TITLE COMPOUNDS

Compound 5(c):



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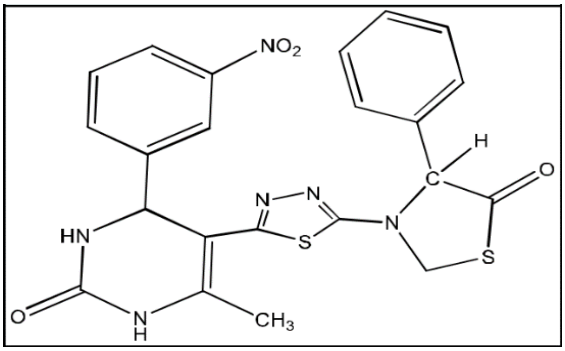
6-Methyl-5-[5-(5-oxo-4-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]-4-(4-nitrophenyl)-3,4- ihydropyrimidin-2(1*H*)-one
Compound 5(d):



6-Methyl-5-[5-(5-oxo-4-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]-4-(3-nitrophenyl)-3,4- ihydropyrimidin-2(1*H*)-one

RESULT:
The objective of this study was to develop novel dihydropyrimidine derivatives associated with thiadiazoles and thiazolidinone goes off, every one of which has a distinct strong pharmacophore, and to study the antibacterial and antifungal effects associated with these molecules.
The objective in the study is to boost the antibacterial activity in dihydropyrimidine derivatives via synthesizing them using thiadiazole and thiazolidinone rings. Due to their resistance to present therapies, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were selected for in vitro antibacterial inquiry. While nitro derivatives studied electron-withdrawing effects, hydroxy/methoxy derivatives were developed for studying electron-donating effects. In addition, derivatives containing thio (sulfur) and oxi (oxygen) have been created with the goal to compare their impact on the pyrimidine ring. 70 various substances were developed, their ability to kill bacteria verified using the spectrum analysis, and finally assessed.

PHYSICAL AND SPECTRAL CHARACTERISTICS
Physical characteristics
Each synthesized biological is a crystalline solid having a light cream to brown shading. Most of the compounds are easily soluble in ethanol and methanol in addition to chloroform. The compounds' melting points varied between 50 to 134° C.

Spectral characteristics
- **IR spectra**

Using KBr disk, the FT-IR 8400S Shimadzu Spectrophotometer was employed to record the infrared spectrum for each substance. In its intended range, each artificial compound showed usual stretching and bending.
- **Mass spectra**

Agilent 6520 was employed to obtain mass spectra (Q-TOF). All the spectra have been collected by direct infusion mass in positive as well as negative mode ionization, ranging from 50 to 500 m/e, utilizing ESI and APCI. Each substance includes a base peak and a M+ molecular ion peak.
- **^{1}H NMR spectra**

A SPECT 400 MHz NMR spectrometer was employed for analyzing the 1H NMR spectra of some of the compounds in CDCl3. Each substance shows an individual chemical shift from TMS in terms of δ ppm. If the δ value comes in the desired range, this confirms the presence of an aromatic ring.

Physical and analytical Characteristics Data
Ethyl 6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate

Com. No.	Com. Name	Mol. formula	Mol. weight (g/mol)	R	X	Melting Point range (°C)	Yield (%)	R_f	Found (Cal.) % N
1(c)	4-Nitro-phenyl-hydrazone	$C_{14}H_{15}N_3O_5$	305.29	4-NO_2	O	210-213	83.4	0.22	13.76 (12.70)
1(d)	3-Nitro-phenyl-hydrazone	$C_{14}H_{15}N_3O_5$	305.29	3-NO_2	O	229-231	84	0.18	13.76 (12.74)

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Ethyl 6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine hydrazine carbothio-amide derivatives

Com. No.	Com. Name	Mol. formula	Mol. weight (g/mol)	R	X	Melting Point range (ºC)	Yield (%)	R_f	Found (Cal.) % N
2(c)	4-Nitro-phenyl thiosemicarbazone	$C_{13}H_{14}N_6O_4S$	350.35	4-NO_2	O	149-151	74	0.30	23.99 (22.71)
2(d)	3-Nitro-phenyl thiosemicarbazone	$C_{13}H_{14}N_6O_4S$	350.35	3-NO_2	O	146-150	73	0.20	23.99 (22.65)

5-(5-amino-1,3,4-thiadiazole-2-yl)-6-methyl-4-substituted-3,4-dihydropyrimidine-2(1H)-one/thione derivatives

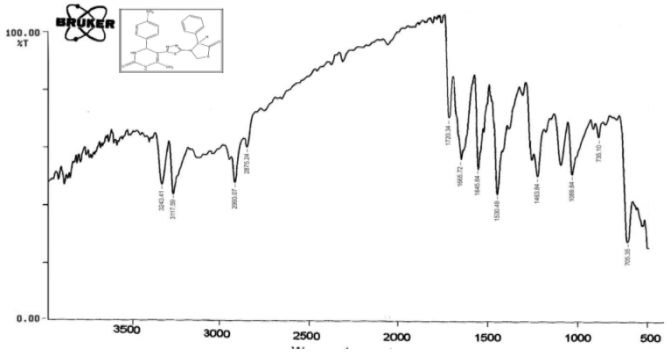
Com. No.	Com. Name	Mol. formula	Mol. weight (g/mol)	R	X	Melting Point range (ºC)	Yield (%)	R_f	Found (Calcd.) % N
3(c)	4-Nitro-6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate	$C_{13}H_{12}N_6O_3S$	332.34	4-NO2	O	223-226	80	0.36	25.29 (25.20)
3(d)	3-Nitro-6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate	$C_{13}H_{12}N_6O_3S$	332.34	3-NO2	O	195-199	75	0.39	25.29 (25.26)

5-(5-(benzylideneamino)-1,3,4-thiadiazole-2-yl)-6-methyl-4-substituted-3,4-dihydropyrimidine-2(1H)-one/thione

Com. No.	Com. Name	Mol. formula	Mol. weight (g/mol)	R	X	Melting Point range (ºC)	Yield (%)	R_f	Found (Calcd.) % N
4(c)	4-Nitro-6-methyl-2-oxo/thioxo-4-aryl -1,2,3,4- tetrahydropyrimidine-5-carboxylate	$C_{20}H_{16}N_6O_3S$	420.44	4-NO_2	O	249-253	77	0.24	19.99 (19.63)
4(d)	3-Nitro-6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate	$C_{20}H_{16}N_6O_3S$	420.44	3-NO_2	O	210-213	79	0.20	19.99 (19.75)

6-methyl-5-[5-(5-oxo-4-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]-4-substituted-3,4-dihydropyrimidin-2(1H)-one/thione

Com. No.	Com. Name	Mol. formula	Mol. weight (g/mol)	R	X	Melting Point range (ºC)	Yield (%)	R_f	Found (Calcd.) % N
5(c)	4-Nitro-6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate	$C_{22}H_{18}N_6O_4S_2$	494.55	4-NO_2	O	310-313	56	0.21	16.99 (16.70)
5(d)	3-Nitro-6-methyl-2-oxo/thioxo-4-substituted-1,2,3,4-tetrahydropyrimidine-5-carboxylate	$C_{22}H_{18}N_6O_4S_2$	494.55	3-NO_2	O	305-309	64	0.31	16.99 (16.83)



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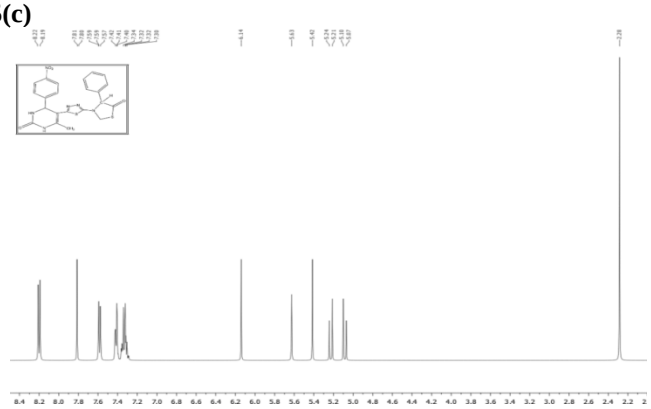
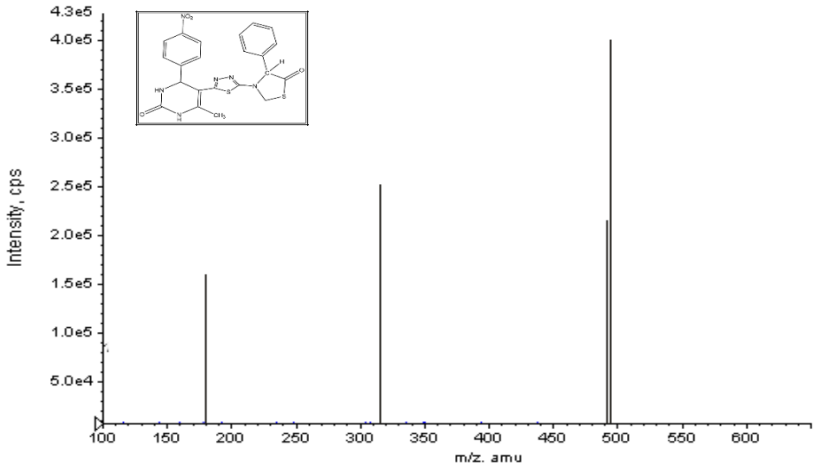
IR spectrum of compound 5(c)

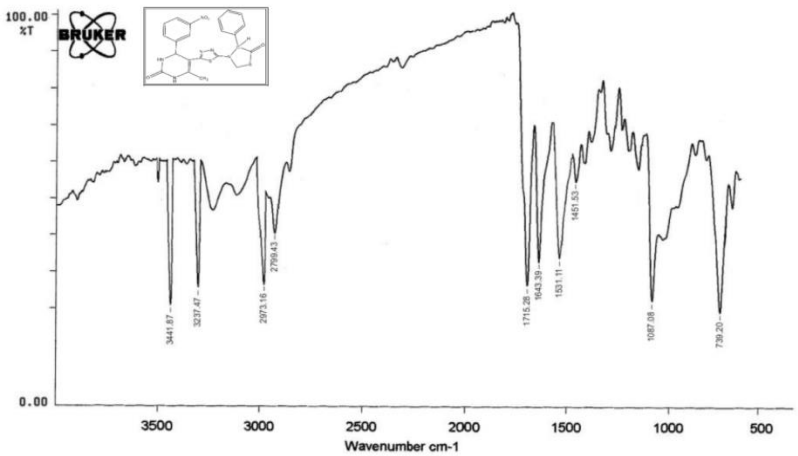


^{1}H NMR spectrum of Compound 5(c)



Mass Spectrum of compound 5(c)

Compound No.	IR (cm^{-1})	^{1}H NMR (δ)	Mass (m/z)
5(c)	3243, 3117 (N-H str.), 2993(C-H str., Ar-H), 2875 (aliphatic C-H str.) 1720 (C=O str. thiazolidinone ring), 1665(>C=O str.in pyrimidinone ring), 1645(C=N of thiadiazole), 1530 (aromatic C-NO2 str.), 1463 (>C=C< conjugated aromatic str.), 1089(>C-N- str.), 735(C-S-C str. thiadiazole ring) 705 (C-S-C str. thiazolidinone ring)	9.25 (s, 1H, -NH), 8.19(d, 2H, Ar-H), 7.83 (s, 1H, -NH), 7.45 (d, 2H, Ar-H), 5.29 (s, 1H, -CH-), 4.83 (s, 1H, -CH), 2.27 (s, 3H, -CH3)	495.07 $[M+1]^{+}$ 494.06 [M] + 316.04 [M-C13H9N5O3S]+· 178.03 [M-C9H8NOS]+·



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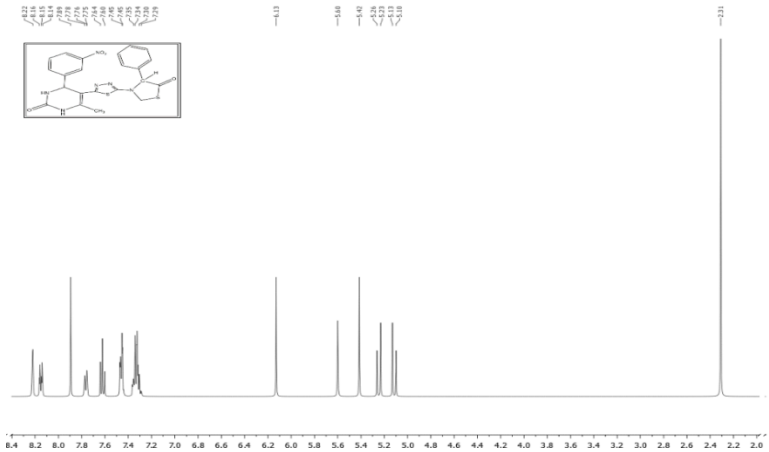
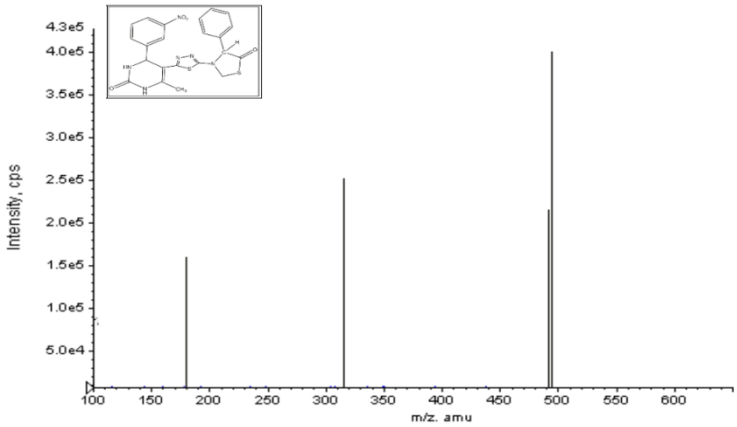
IR spectrum of compound 5(d)





^{1}H NMR spectrum of Compound 5(d)

Mass Spectrum of compound 5(d)

Compound No.	IR (cm^{-1})	^{1}H NMR (δ)	Mass (m/z)
5(d)	3441, 3237 (N-H str.), 2973(C-H str., Ar-H), 2799 (aliphatic C-H str.) 1715 (C=O str. thiazolidinone ring), 1663(>C=O str.in pyrimidinone ring), 1643(C=N of thiadiazole), 1531 (aromatic C-NO_2 str.), 1451 (>C=C< conjugated aromatic str.), 1087(>C-N-str.), 739 (C-S-C str. thiadiazole ring) 685(C-S-C str. thiazolidinone ring)	9.33(s, 1H, -NH) 7.77(s, 1H, -NH) 7.61(m, 4H, Ar-H) 5.39(s, 1H, -CH-) 4.89(s, 1H, -CH), 2.29(s, 3H, $-CH_3$	495.07 $[M+1]^+$ 494.06 $[M]^{+\cdot}$ 316.04 $[M-C13H9N5O3S]^{+\cdot}$ 178.03 $[M-C9H8NOS]^{+\cdot}$

ANTIMICROBIAL ACTIVITY:
ANTIBACTERIAL ACTIVITY:

Using the cup plate method, the in vitro antimicrobial activity of the synthesized compounds (5c-5d) was evaluated against two gram-negative bacteria, Pseudomonas aeruginosa and Escherichia coli, and one gram-positive bacteria, Staphylococcus aureus. Cefuroxime and ciprofloxacin are commonly used antibacterial agents. In Petri dishes, pH 6.8 nutrient agar was solidified, and the test organisms were introduced onto the agar. Using a cork borer, wells were drilled and stock solutions of the synthesized compounds and standards were added. The stock solutions were created in dimethyl formamide (DMF) at concentrations of 50 µg/ml and 25 µg/ml. The zones of inhibition were assessed when the plates were incubated for 24 hours at 37°C. Tables and graphs with DMF as the control show the results, including inhibition percentages.

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Zone of inhibition in mm of *in vitro* antibacterial activity of the synthesized compounds 5c-5d

Sample	***E. coli* (Gram - Ve bacteria)**		***P. aeruginosa* (Gram - Ve bacteria)**		***S. aureus* (Gram + Ve bacteria)**	
Concentration in µg/ml	**25**	**50**	**25**	**50**	**25**	**50**
Ciprofloxacin	23	28	23	24	19	21
Cefuroxime	22	27	22	23	20	21
5(c)	15	17	14	16	13	15
5(d)	14	15	14	16	14	16

Zone of inhibition in percentage of *in vitro* antibacterial activity of the synthesized compounds 5c-5d

Sample		***E. coli***		***P. aeruginosa***		***S. aureus***	
Concentration in µg/ml		**25**	**50**	**25**	**50**	**25**	**50**
Ciprofloxacin		82%	97%	84%	94%	88%	93%
Cefuroxime		89%	98%	93%	96%	90%	93%
5(c)		55%	62%	46%	54%	54%	59%
5(d)	48%	55%	47%	52%	48%	56%	

Compound c **Compound d**

DISCUSSION:

The research aimed to synthesize novel dihydropyrimidine derivatives that incorporate thiadiazole and thiazolidinone rings, driven by the goal of enhancing their antibacterial and antifungal activities. A thorough literature review indicated that these compounds exhibit a range of pharmacological properties, making them promising candidates for addressing the challenges posed by resistant bacterial strains, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Additionally, the antifungal potential was evaluated against key pathogens such as Candida albicans, Aspergillus niger, and Aspergillus clavatus. The study also delved into structure-activity relationships by synthesizing derivatives with various substituents: hydroxy and methoxy groups were introduced to explore electron-donating effects, while nitro groups were incorporated to investigate the impact of electron-withdrawing characteristics. Furthermore, oxo and thio derivatives were created to assess the influence of oxygen and sulfur on the biological activity of the compounds. In total, 70 unique compounds were synthesized and characterized using spectral analysis, yielding valuable insights into their structural properties and potential therapeutic applications. This work contributes significantly to the search for new antimicrobial agents in the context of rising microbial resistance, highlighting the importance of innovative drug development strategies.

SUMMARY:

This research successfully synthesized a series of novel dihydropyrimidine derivatives featuring thiadiazole and thiazolidinone rings, demonstrating promising potential for antibacterial and antifungal activities. The systematic exploration of structure-activity relationships through the introduction of various substituents has provided insights into how these modifications can enhance pharmacological properties. The synthesized compounds exhibited significant

efficacy against resistant bacterial strains and key fungal pathogens, suggesting their potential as new therapeutic agents. As microbial resistance continues to pose a major challenge in clinical settings, the findings of this study contribute to the development of innovative solutions for effective infection management. Future work will involve further biological evaluations and optimization of these compounds to realize their full therapeutic potential.

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