Research

MICROWAVE-ASSISTED SYNTHESIS, MOLECULAR DOCKING, AND ANTIMICROBIAL EVALUATION OF NOVEL 1, 2, 3, 4-TETRAHYDROPYRIMIDINE DERIVATIVES

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ABSTRACT

The emergence of antimicrobial resistance necessitates the development of novel chemo types with improved efficacy and favorable pharmacokinetic properties. In the present study, a number of novel 1, 2, 3, 4-tetrahydropyrimidine derivatives were created, produced, described, and assessed physiologically as possible antibacterial agents. A total of 24 derivatives were rationally designed and screened using in silico approaches, including physicochemical profiling, ADME prediction, toxicity assessment, target prediction, and molecular docking studies against selected bacterial and fungal targets. Among these, six lead compounds (D1–D6) demonstrating favorable drug-likeness and binding affinity were synthesized via a microwave-assisted Biginelli multicomponent reaction. Compared with conventional synthesis, microwave irradiation significantly reduced reaction time and improved product yields (70–85%). Structural characterization was performed using TLC, UV spectroscopy, FTIR, ^{1}H-NMR, and mass spectrometry. Docking analysis revealed promising binding interactions of selected derivatives against antimicrobial targets, particularly compounds D3 and D4, which demonstrated enhanced affinity toward bacterial and fungal proteins. Antimicrobial study in vitro against Candida albicans, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus revealed detectable action with minimum inhibitory values between 4 and 8 mg/mL. Compound D3 exhibited notable activity against *E. coli* with inhibition comparable to the reference drug norfloxacin, while D4 displayed broad-spectrum antibacterial and antifungal potential. ADME and toxicity predictions further indicated acceptable pharmacokinetic behavior and low predicted toxicity profiles. Overall, the findings identify tetrahydropyrimidine derivatives, particularly D3 and D4, as promising scaffolds for further optimization toward the development of novel antimicrobial agents.

Keywords: Antimicrobial activity, Biginelli reaction, computer-aided drug design, docking studies, microwave-assisted synthesis, pyrimidine derivatives, structure–activity relationship, tetrahydropyrimidines, etc.

INTRODUCTION

However, the discovery and development of new drugs continue to be important in finding solutions for new threats to health care and in solving problems that are linked to existing medications. Drug development is an elaborate and multi-disciplinary process that entails lead identification, optimization, pre-clinical evaluation and clinical trial to create safe and effective therapeutic agents[1]. Nevertheless, conventional drug development takes several years of efforts before reaching market success since it is time consuming and costly. For that reason, modern pharmaceutical research relies increasingly on computational approaches and new synthetic techniques in order to speed up the discovery process and make it more efficient[2]. Computer-aided drug discovery (CADD) has proven to be an excellent approach in modern medicinal chemistry that helps with rational drug discovery using computational methods and models. The use of computer-aided drug design involves different computational approaches such as structure-based and ligand-based drug discovery to create new molecular compounds for therapeutic purposes[3]. The virtual screening technique, which involves screening of a large number of compounds from a chemical database in search for new active compounds through various computational methodologies such as molecular modeling, pharmacophore analysis and docking techniques, has contributed tremendously to drug discovery processes[4]. Molecular docking, in particular, has become a widely applied computational strategy for studying ligand–receptor interactions and predicting binding affinity, thereby supporting the identification of promising therapeutic candidates[5].

Microwave organic synthesis has been receiving much attention as one of the efficient and environmentally-friendly methods in synthetic chemistry. In comparison with traditional heating procedures, microwave radiation ensures fast and homogeneous heating process leading to shorter reaction time, higher yield, increased selectivity and decreased use of solvents[6]. It is known that microwave-assisted reactions possess many benefits such as high energy efficiency, low by-product formation and green character of chemistry[7]. Thus, this technique becomes more widely used in medicinal chemistry to synthesize various bioactive heterocyclic molecules. There are a number of different heterocyclic structures; one of them is a pyrimidine ring, which derivatives show wide pharmacological activity[8]. Pyrimidines can be described as a nitrogen containing six-membered aromatic heterocycle, which is an integral part of nucleic acids and plays a very important role in biological system[9]. Derivatives of pyrimidine proved to possess antimicrobial, antiviral, anticonvulsant, antihypertensive, antimalarial and anti-inflammatory activities. Especially, interest is focused on 1,2,3,4-tetrahydropyrimidine derivatives. [10,11].

The Biginelli reaction is known as an efficient multicomponent synthetic method for the synthesis of various dihydropyrimidines. In this regard, Biginelli reaction is considered as an interesting route towards the synthesis of different pyrimidine derivatives using a condensation process between β-ketoesters, aldehydes, and urea/thiourea derivatives[12]. Because of the advantages of the Biginelli reaction including high efficiency and high adaptability of the reaction to different conditions, the reaction can be performed under the microwave irradiation condition[13]. One of the major challenges facing medicine today is microbial resistance that limits the use of antibiotics[14]. Because of increasing microbial resistance to drugs, the development of novel antimicrobial agents with higher potency and less resistance rate has become an important issue[15]. Pyrimidine derivatives are capable of showing high antimicrobial activity through different mechanisms of actions[16]. Consequently, using microwave irradiation, pyrimidine chemistry, and drug designing techniques for producing novel tetrahydropyrimidines with improved antimicrobial activity is a useful strategy.

MATERIALS AND METHODS

Designed 1, 2, 3, 4-tetrahydropyrimidine derivatives

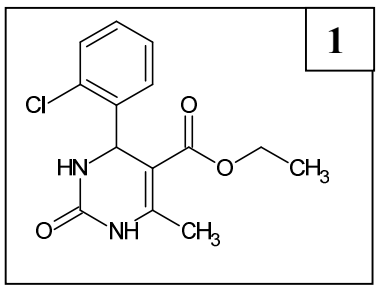
Designed 24 derivatives based on the proposed procedure. The 24 designed molecules were developed using ChemSketch software. The physical properties of each molecule, including its composition, molar refractivity, and density, among others, were determined using ChemSketch software. PubChem, ZINC, and other databases were used to confirm the uniqueness of the 24 designed compounds.[17].

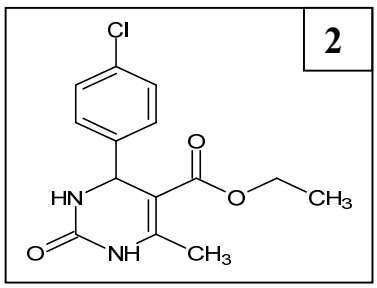
Table 1*: List of designed 1,2,3,4-tetrahydropyrimidine derivatives*

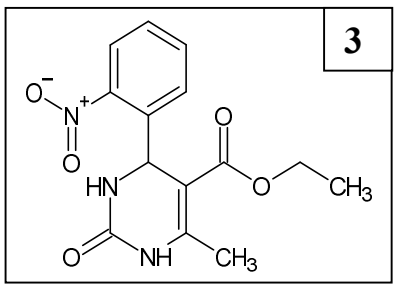
Sr. No	Compound No.	Compound name	SMILES
1	Compound-1	Ethyl 4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=O)NC1C2=CC=CC=C2Cl)C
2	Compound-2	Ethyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CC=1NC(=O)NC(C2CCC(CL)CC2)C=1C(=O)OCC
3	Compound-3	Ethyl 6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=O)NC1C2=CC=CC=C2[N+](=O)[O-])C
4	Compound-4	Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=O)NC1C2=CC(=CC=C2)[N+](=O)[O-])C
5	Compound-5	Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=O)NC1C2=CC=CC=C2[N+](=O)[O-])C
6	Compound-6	Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbox	CCOC(=O)C1=C(NC(=O)NC1C2=CC=CC=C2)C
7	Compound-7	Ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=S)NC1C2=CC=CC=C2Cl)C
8	Compound-8	Ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	S=C1NC(C2CCC(CL)CC2)C(=C(C)N1)C(=O)OCC
9	Compound-9	Ethyl 6-methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=S)NC1C2=CC=CC=C2[N+](=O)[O-])C
10	Compound-10	ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=S)NC1C2=CC(=CC=C2)[N+](=O)[O-])C
11	Compound-11	Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=S)NC1C2=CC=C(C=C2)O)C
12	Compound-12	Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CCOC(=O)C1=C(NC(=S)NC1C2=CC=CC=C2)C
13	Compound-13	Methyl 4-(2-chlorophenyl)-6-	CC1=C(C(NC(=O)N1)C2

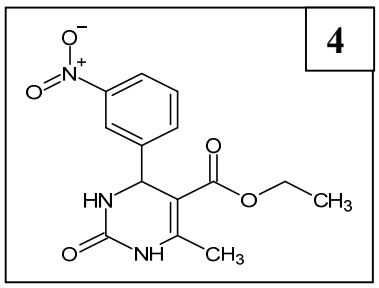
		methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	=CC=CC=C2Cl)C(=O)OC
14	Compound-14	Methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=O)NC1C1CCC(CL)CC1
15	Compound-15	Methyl 6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	O=[N+]([O-])C1CCCCC1C1NC(=O)NC(C)=C1C(=O)OC
16	Compound-16	Methyl 6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	O=[N+]([O-])C1CC(CCC1)C1NC(=O)NC(C)=C1C(=O)OC
17	Compound-17	Methyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=O)NC1C1CCC(O)CC1
18	Compound-18	Methyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=O)NC1C1CCCCC1
19	Compound-19	Methyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=S)NC1C1CCCCC1CL
20	Compound-20	Methyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=S)NC1C1CCC(CL)CC1
21	Compound-21	Methyl 6-methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	S=C1NC(C2CCCCC2[N+]([O])=O)C(=C(C)N1)C(=O)OC
22	Compound-22	Methyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CC1=C(C(NC(=S)N1)C2=CC(=CC=C2)[N+](=O)[O-])C(=O)OC
23	Compound-23	Methyl 4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	COC(=O)C1=C(C)NC(=S)NC1C1CCC(O)CC1
24	Compound-24	Methyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	CC1=C(C(NC(=S)N1)C2=CC=CC=C2)C(=O)OC

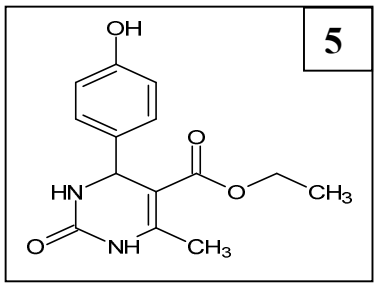
- **Chemsketch software was used to draw the 24 designed compounds.**

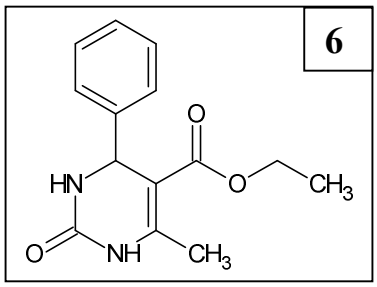


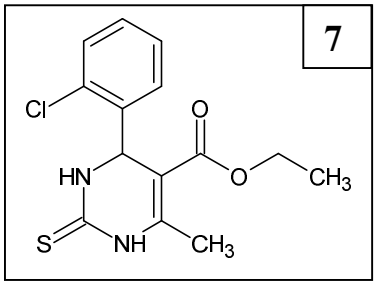








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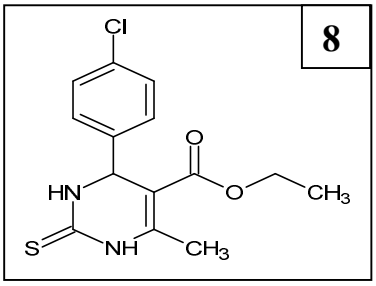
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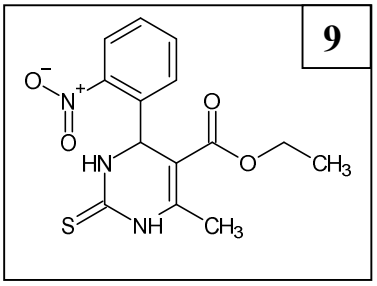
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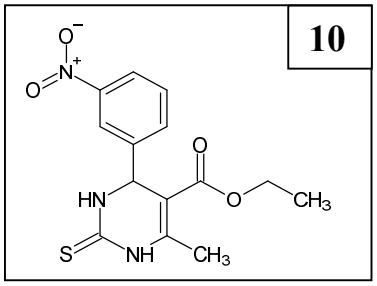
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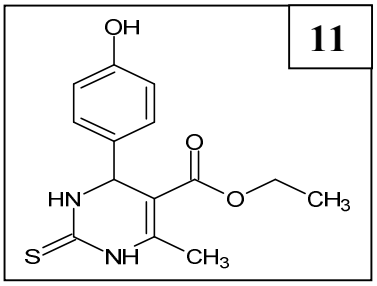
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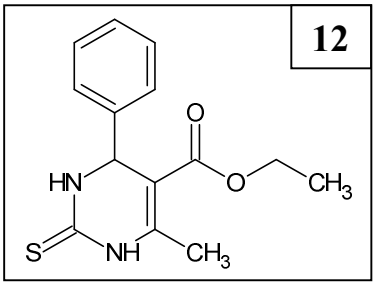
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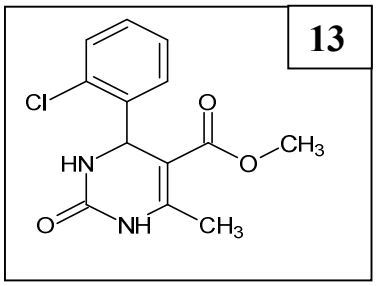
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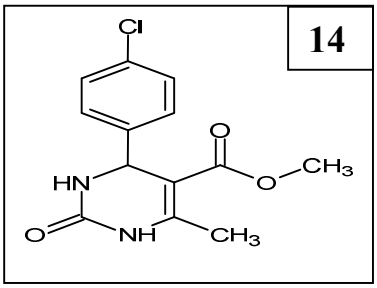
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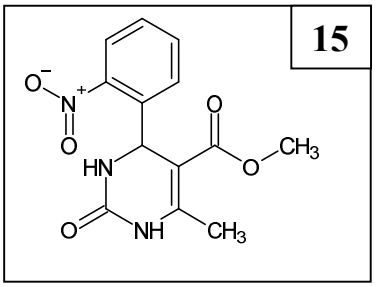
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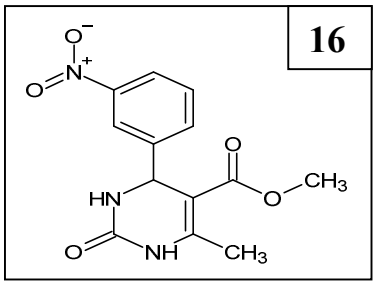
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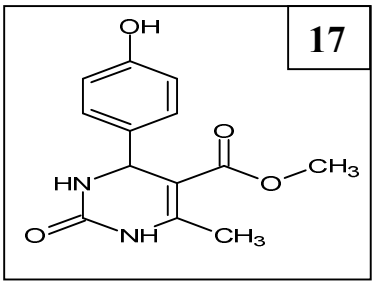
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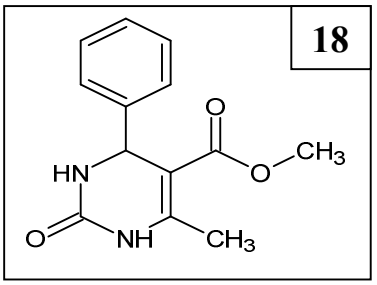
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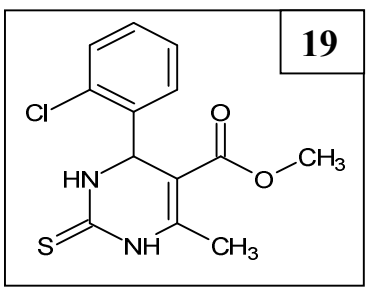
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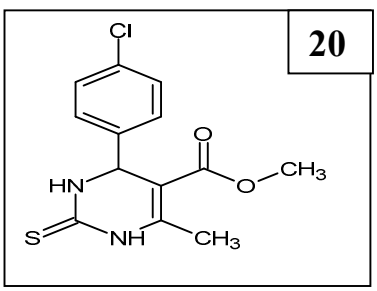
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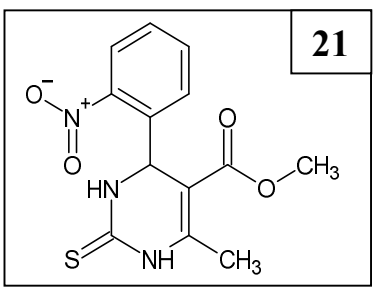
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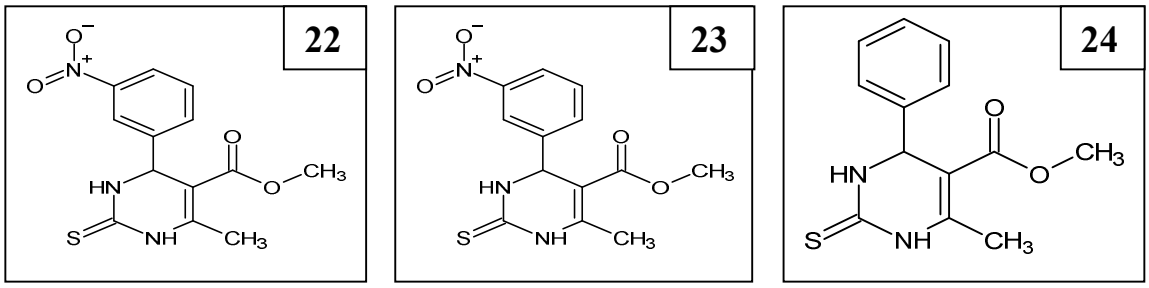


ADME and Toxicity Prediction

The drug-likeness properties and safety concerns of the designed molecules were examined by utilizing the computational prediction methods to study their drug-likeness and pharmacokinetic and toxicological properties. The ADME properties were analyzed using the online platform of ADMETlab 2.0, where predictions are generated regarding the physicochemical, pharmacokinetic properties, medicinal chemistry friendliness, and drug-likeness of the molecular entities. This allows for efficient drug design at an early stage by rapidly analyzing potential molecules. [18].

The toxicity assessment was conducted through the virtual laboratory PROTOX 3.0. Acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, toxicity pathways, and toxicity targets are just a few of the toxicity endpoints that it may predict. The major criteria for toxicity assessment were LD50 values, expressed in mg/kg body weight, that divide drugs by their toxicity, which is described by global classifications from highly toxic to non-toxic. The ADME analysis combined with toxicity prediction helps greatly to estimate the safety and efficacy of drugs, thus reducing costs and number of animals used in the experiments. [19].

Target Prediction

Target prediction of designed compounds was done using the Swiss target prediction database. The target prediction database was able to predict the possible interaction between the designed compounds and the target protein successfully. Using strong algorithms and an extensive data set, the target prediction database was able to predict effectively. [20].

Molecular Docking Studies

Step 1: Ligand Preparation:

The structures of 24 compounds were designed using ChemSketch and saved in SDF format. Energy minimization and geometry optimization were performed using Avogadro, followed by conversion of optimized structures into PDB format using UCSF Chimera 1.18 and AutoDock Vina-compatible tools. The converted files included appropriate atom types and partial charges required for molecular docking studies[21].

Step 2: Receptor Preparation:

Protein structures of chosen bacterial and fungal target receptors were obtained from the Protein Data Bank (PDB), which included Gram-positive, Gram-negative, and fungal receptors. Protein preparation involved the use of Discovery Studio Visualizer, where co-crystallized ligands, water molecules, and

non-canonical amino acids were removed to obtain prepared receptor structure in the form of PDB file. [22].

Step 3: Receptor–Ligand Docking Interaction:

The molecular docking was carried out using software tools such as PyRx, myPresto Portal, and UCSF Chimera 1.18 to investigate receptor-ligand interaction. Suitable parameters such as grid dimensions and grid center were specified to ensure that the ligands bind and sample conformations in the receptor pocket. [23].

Step 4: Visualization:

The docked complexes were analyzed and visualized using Discovery Studio Visualizer for both two-dimensional (2D) and three-dimensional (3D) molecular interaction studies[24].

General Synthetic Procedure for 1, 2, 3, 4-tetrahydropyrimidine derivatives

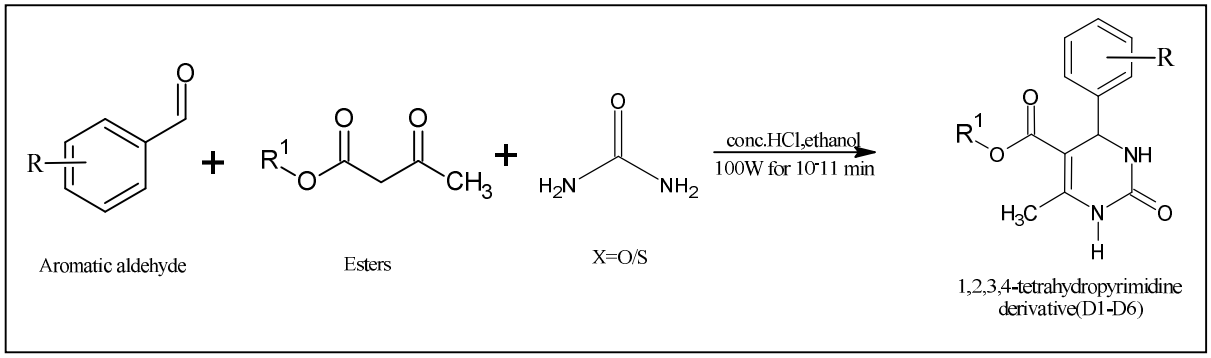


Figure 1: *Synthetic scheme of 1, 2, 3, 4-tetrahydropyrimidine*

Using the conventional method: A mixture of urea, ethyl acetoacetate, and aromatic aldehyde (0.1 mol) was prepared using equal molar quantities. During the preparation process, a catalytic quantity of concentrated HCl (1 ml) was introduced into this mixture, which was allowed to undergo reflux for 3 hours. The blend was allowed to cool before being kept in the fridge overnight. The resulting solid was filtered. Further heating for 1 hour 30 minutes was allowed in a water bath. [25]

Microwave-assisted method: A solution of urea, ethyl acetoacetate, and aromatic aldehyde (0.1 mol each in equimolar amounts) was prepared using 25 ml of ethanol. Appropriate catalytic quantities of concentrated HCl (1 ml) were added to the solution and the solution was treated in a microwave oven for 4-5 minutes at 100W.Cooling and overnight storage in the refrigerator followed. The solid was filtered out. The filtrate was further processed in the oven for 3-4 minutes. The solid obtained was again separated through cooling and filtration process.

Identification and Characterization Methods:

The synthesized compounds were purified using recrystallization technique to determine the percentage yield. Structure elucidation was done via physical chemical methods and spectroscopic techniques such as melting point analysis, thin-layer chromatography (TLC), UV spectroscopy, FTIR, ^{1}H NMR, and mass spectrometry. [26,27,28].

The melting points were taken by open capillary technique using Kumar melting point apparatus without any correction [29]. The TLC spots were identified by running them on pre-coated silica gel plate with ethyl acetate: n-hexane (2:8) as mobile phase and calculating their respective Rf value [30]. The UV spectrum was taken by Shimadzu UV spectrophotometer whereas the FTIR spectrum was recorded with Shimadzu FTIR spectrometer. Further structural characterization was done by ^{1}H NMR spectroscopy with DMSO/$CDCl_3$ as solvent and molecular mass was confirmed by LC-MS spectroscopy.

Biological Evaluation – Antimicrobial Activity:

The antibacterial and antifungal behaviors of the synthesized derivatives were tested against some chosen bacteria and fungi using the MIC and well diffusion methods. The test organisms employed were S. aureus, E. coli, Bacillus spp., and C. albicans. Bacterial and fungal studies were done on nutrient agar and sabouraud dextrose agar respectively. Norfloxacin and miconazole were used as antibacterial and antifungal agents respectively. [31].

The stock solution for the synthesized compounds and standard drugs was made in DMSO at a concentration of 10 mg/mL. The media sterility was also tested using control before performing biological analysis.

In order to conduct **MIC determination**, serial dilutions of the test compound were made in nutrient broth medium which was then inoculated with selected microorganisms and allowed to grow under controlled conditions. The MIC of the compounds was determined as the minimum concentration of the compounds which could prevent the growth of microbes after incubating for 24 hours in the case of microorganisms and for 48 hours in case of fungi[32].

Antimicrobial screening was carried out using well diffusion technique. Inoculated agar medium was used to prepare Petri plates. Wells were made in the Petri plates using sterile borer. Test compounds were placed in the well and allowed to incubate under optimized condition for antimicrobial activity. Zone of inhibition in millimeters was observed to check the activity of antimicrobial agents. [33,34].

RESULTS AND DISCUSSION

Physicochemical Characterization

Lipinski's rule was used to assess the physicochemical properties of all the designed 1, 2, 3, 4-tetrahydropyrimidine derivatives and predict their drug-like properties.

Table 2: *Physicochemical characterization of designed compound*

Compound	Physicochemical Properties						Drug-Likeness Rules
	TPSA (A^0)	M.W (g/mol)	n-ROT	Log P	n-HA	n-HD	Lipinski
	(0-140)	(100-500)	(0-11)	(0-5)	(0-12)	(0-7)	
Compound 1	**67.43**	**294.73 g/mol**	**4**	**3.19**	**5**	**2**	**Follow**
Compound-2	**67.43**	**294.73 g/mol**	**4**	**3.19**	**5**	**2**	**Follow**
Compound-3	**125.845**	**305.29 g/mol**	**4**	**1.78**	**5**	**2**	**Follow**
Compound-4	**125.845**	**305.29 g/mol**	**4**	**1.78**	**5**	**2**	**Follow**
Compound-5	**87.66**	**278.87 g/mol**	**4**	**2.24**	**6**	**2**	**Follow**
Compound-6	**111.192**	**260.29 g/mol**	**4**	**1.87**	**3**	**2**	**Follow**
Compound-7	127.692	310.8 g/mol	4	2.69	3	2	Follow
Compound-8	127.692	310.8 g/mol	4	2.69	3	2	Follow
Compound-9	132.043	321.35g/mol	4	1.95	5	2	Follow
Compound-10	132.043	321.35 g/mol	4	1.95	5	2	Follow
Compound-11	122.183	292.36 g/mol	4	1.74	4	3	Follow
Compound-12	117.389	276.35 g/mol	4	2.04	3	2	Follow

Compound-13	115.13	280.70 g/mol	2	2.14	3	2	Follow
Compound-14	115.13	280.70 g/mol	2	2.14	3	2	Follow
Compound-15	119.48	291.26 g/mol	3	1.39	5	2	Follow
Compound-16	119.48	246.26 g/mol	3	1.39	5	2	Follow
Compound-17	262.26	291.26 g/mol	2	1.19	4	3	Follow
Compound-18	104.82	262.26 g/mol	2	1.48	3	2	Follow
Compound-19	121.32	296.76 g/mol	2	2.30	3	2	Follow
Compound-20	121.32	296.76 g/mol	2	2.30	3	2	Follow
Compound-21	125.67	307.32 g/mol	3	1.56	5	2	Follow
Compound-22	125.67	307.32 g/mol	3	1.56	5	2	Follow
Compound-23	115.81	278.32 g/mol	2	1.35	4	3	Follow
Compound-24	111.02	262.37 g/mol	2	1.65	3	2	Follow

All the designed compound follows the Lipinski rule. The Lipinski rule was used to assess the physicochemical properties of designed compound. The study was extended further for target prediction and molecular docking of 24 designed compound.

ADME Study of synthesized derivatives

The assessment of ADME for the derivatives was carried out using ADMET lab 2.0 and pkCSM tool. It is very well known that absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of chemicals need to be estimated at an early stage. The above-mentioned knowledge may be used to predict the results of ADME of a synthesized compound using web server like ADMET lab 2.0.

Table 3: *The ADME study of derivatives (D1-D6)*

Compound	**Absorption (Intestinal human absorption)**	**Distribution**			**Metabolism**	**Excretion**	
		PPB (%)	**VD (L/kg)**	**BBB**	**CYP1A2 inhibitor**	**CL (ml/min/kg)**	**T1/2 (Hr)**
Norfloxacin	**98.96**	**67.87**	**2.77**	**No**	**Yes**	**0.85**	**0.12**
Miconazole	**92.67**	**99.66**	**3.11**	**No**	**Yes**	**0.78**	**0.06**
D1	91.85	92.52	0.78	No	Yes	0.107	0.22
D2	90.373	92.64	0.73	No	Yes	0.048	0.17
D3	80.441	88.80	0.64	No	Yes	0.502	0.45
D4	80.323	87.51	0.75	No	Yes	0.543	0.31
D5	91.085	80.02	0.55	No	Yes	0.419	0.73
D6	93.57	81.92	0.67	No	Yes	0.573	0.14

From the ADME Evaluation, all the derived derivatives (D1-D6) exhibit high absorption properties while at the same time having high binding with plasma proteins; hence, they do not permeate the blood-brain barrier. These derivatives undergo metabolic process via CYP1A2 enzyme but with various clearance rates with short half-life of all derivatives.

Toxicity study of synthesized derivatives

ProTox 3.0 is a computer laboratory used in the prediction of molecular toxicity. Toxicity prediction is one of the most important parts of drug development. Not only will it be faster than identifying the toxic dose through animals, but it will also minimize the number of animal experiments that need to be done. The ProTox 3.0 program will facilitate in the prediction of organ toxicity, which includes

hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, and so forth. ProTox 3.0 software predicts the synthesized derivatives in the following manner:

Table 4: *Toxicity study of synthesized derivatives (D1-D6)*

Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	LD_{50} mg/Kg
Norfloxacin	**Inactive**	**Inactive**	**Inactive**	**-**	**2500**
Miconazole	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**519**
D1	Inactive	-	Inactive	Inactive	630
D2	Inactive	-	Inactive	Inactive	1644
D3	Inactive	Inactive	Inactive	-	650
D4	Inactive	Inactive	Inactive	-	785
D5	Inactive	-	Inactive	Inactive	2595
D6	Inactive	-	Inactive	Inactive	2495

There have no Hepatotoxicity, Carcinogenicity, Immunotoxicity and Mutagenicity for synthesized derivatives, and all derivatives belong to toxicity classes 4 and 5; therefore, they are nontoxic and non-irritant when compared with standard compounds, having greater LD50 values.

Target Prediction

The target prediction by using Swiss target prediction. The Design compounds 1, 2, 3, 4-tetrahydropyrimidine derivatives.

1)PDB ID-4URM

Crystal Structure of Staph GyraseB 24kDa in complex with Kibdelomycin.

- Classification: ISOMERASE
- Mutation(s): No
- Method: X-RAY DIFFRACTION
- Resolution: 2.94 Å

2) PDB ID-6A4M

Structure of urate oxidase from Bacillus subtilis 168.

- **Classification: HYDROLASE**
- Mutation(s): No
- Method: X-RAY DIFFRACTION
- Resolution: 2.60 Å

3) PDB ID-3FVZ

Crystal Structure of E. coli Topoisomerase IV co-complexed with inhibitor.

- **Classification: ISOMERASE**
- Mutation(s): No
- Method: X-RAY DIFFRACTION
- Resolution: 1.80 Å

4) PDB ID-6AKZ

Crystal structure of GlcNAc Inducible Gene 2, GIG2 (DUF1479) from Candida albicans

- Classification: OXIDOREDUCTASE
- Mutation(s): No
- Method: X-RAY DIFFRACTION
- Resolution: 1.69Å

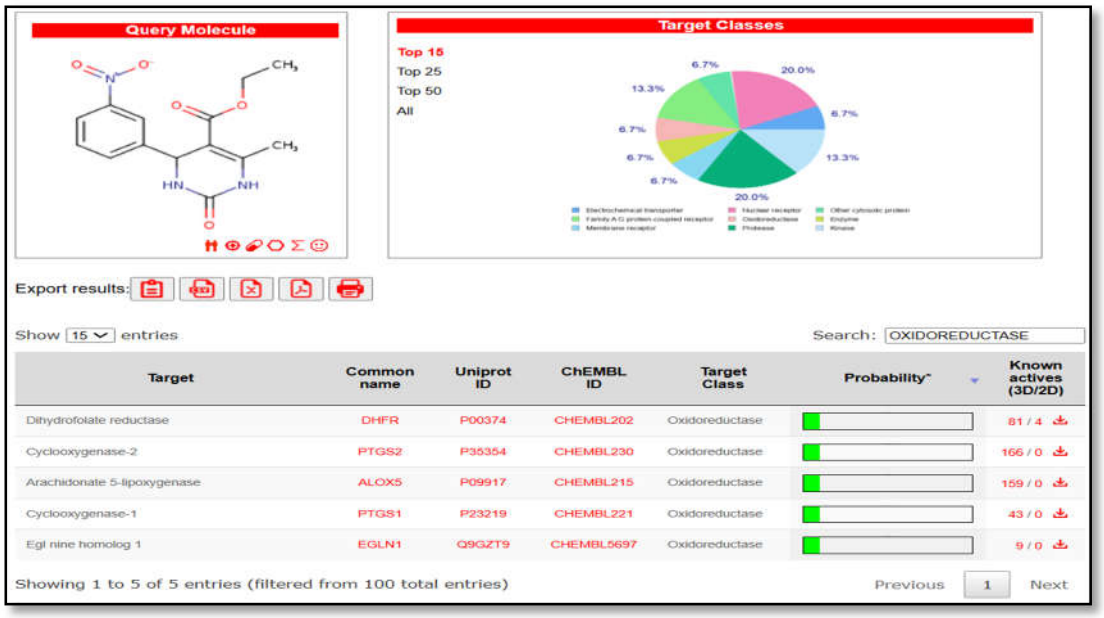


Figure 2: *Target prediction of towards target protein*

Molecular Docking Study

AutoDock Vina software is used for conducting molecular docking analysis on all the designed 1,2,3,4-tetrahydropyrimidine derivatives for antimicrobial studies. Crystal structure of staph gyrase B 24KDa in complex with Kibelomycin from Staphylococcus aures (PDB ID = 4URM). Urate oxidase from Bacillus substilis 168 (PDB ID = 6A4M). Crystal structure of E. coli Topoisomerase IV co-complexes with inhibitor from Escherichia coli (PDB ID = 2FV5). Crystal structure of GlcNac inducible Gene2, G1G2 (DUF147

Table 5: *Molecular docking Score of 24 Designed compound*

Sr.No	Compound	Docking Score (Kcal/mol)			
		Gram-positive bacteria		Gram-negative bacteria	Fungi
		S. aureus (4URM)	B. subtilis (6A4M)	E. coli (3FV5)	C. albicans (6AKZ)
*	*Norfloxacin*	*-7.2*	*-7.8*	*-6.2*	-
*	*Miconazole*	-	-	-	*-7.6*
1	Compound-1 (D1)	-6.8	-6.7	-5.8	-6.9
2	Compound-2 (D2)	-6.2	-5.7	-6.2	-6.8
3	Compound-3 (D3)	-7	-6.7	-6	-7.2
4	Compound-4 (D4)	-8.5	-7.2	-7.1	-7.7
5	Compound-5 (D5)	-6.3	-6.7	-6.3	-6.8
6	Compound-6 (D6)	-7.9	-6.4	-6.6	-6.8

7	Compound-7	-5.3	-6.7	-5.9	-6
8	Compound-8	-5.3	-6.9	-5.5	-5.8
9	Compound-9	-5.9	-6.6	-5.7	-6.3
10	Compound-10	-6.6	-7.3	-5.8	-6.8
11	Compound-11	-6.1	-6.9	-5.5	-6
12	Compound-12	-6.3	-6.5	-5	-6.3
13	Compound-13	-6.8	-6.8	-5.9	-6.7
14	Compound-14	-6	-6.8	-5.5	-6
15	Compound-15	-5.9	-6.7	-6	-7.2
16	Compound-16	-7.4	-7.7	-6.3	-7.3
17	Compound-17	-6.4	-6.8	-5.7	-6.8
18	Compound-18	-6.4	-6.7	-6.5	-6.9
19	Compound-19	-6.1	-6.7	-5.7	-6.1
20	Compound-20	-6.1	-6.4	-5.1	-6
21	Compound-21	-6.3	-6.3	-5.9	-6.6
22	Compound-22	-6.7	-6.7	-5.1	-6.2
23	Compound-23	-6	-6	-5.6	-6.5
24	Compound-24	-6	-5.6	-5.3	-6

The developed ligands D1, D2, D3, D4, D5, and D6 have exhibited favourable binding properties against receptor 4URM, 6A4M, and 2FV5, which are antibacterial, and 6AKZ for antifungal property compared to the benchmark drugs Norfloxacin and Miconazole respectively.

Synthetic Yield and Characterization

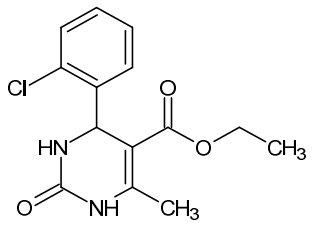
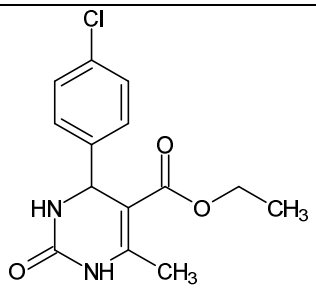
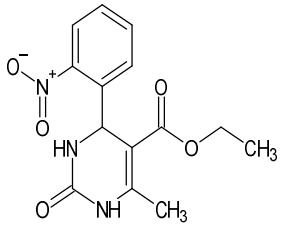
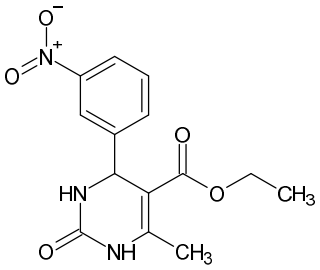
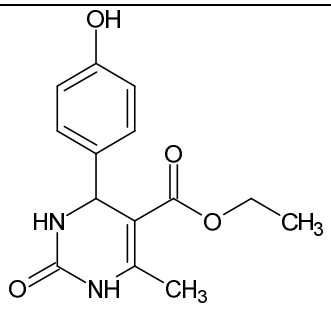
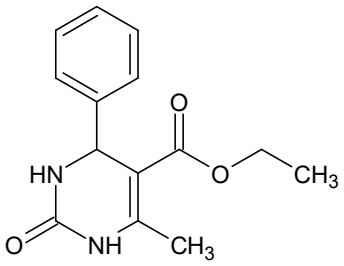
After carrying out the in-silico analysis of the design compound, the top six compounds were synthesized for more analysis. Six compounds of 1, 2, 3, 4-tetrahydropyrimidine derivatives were successfully synthesized by the procedure outlined above and further purified by the use of ethanol recrystallization technique, with their purity analyzed by thin-layer chromatography. Physical properties of the synthesized derivatives are presented below:

Structure molecular formula, molecular weight, percentage yield, melting point & Rf Value (TLC) λ-max(mm)

Table 6: *Physicochemical properties of synthesized derivatives (D1-D6)*

Comp.	M. F	M.W g/mol	Conventional		Microwave		R_f value	λ -max (nm)
			(%) Yield	M.P (^{0}C)	(%) Yield	M.P (^{0}C)		
D1	$C_{14}H_{15}ClN_2O_3$	294.73	55	192-194	75	193-195	0.79	310
D2	$C_{14}H_{15}ClN_2O_3$	294.73	56	193-195	72	192-193	0.70	310
D3	$C_{14}H_{15}N_3O_5$	305.29	67	122-124	70	123-122	0.69	296
D4	$C_{14}H_{15}N_3O_5$	305.29	60	120-122	85	117-120	0.65	296
D5	$C_{14}H_{16}N_2O_4$	276.87	54	220-222	75	220-223	0.62	283
D6	$C_{14}H_{15}N_2O_3$	260.29	50	188-190	79	189-191	0.70	295

Table 7: *Structure of synthesized D1-D6 Derivatives*

 Compound-D1	 Compound-D2	 Compound-D3
 Compound-D4	 Compound-D5	 Compound-D6

Spectral Characterization of Synthesized Compound D4

Synthesized compound D4 ($C_{14}H_{15}N_3O_5$; molecular weight of 305.29 g/mol) was analyzed by means of FTIR, ^{1}H NMR, and ESI–MS techniques in order to establish the characteristic structural and molecular properties of the compound under investigation. FTIR spectrum analysis showed distinct absorption bands that were attributed to N–H/O–H stretching (3485–3176 cm^{-1}), aliphatic C–H stretching (2991–2929 cm^{-1}), and carbonyl functionality (C=O; around 1710 cm^{-1}). Moreover, a peak near 1664 cm^{-1} corresponded to conjugated carbonyl and/or C=N groups. Structural characteristics of the compound D4 were further proven by analyzing the ^{1}H NMR spectrum of the compound. It is worth mentioning that ^{1}H NMR spectrum analysis showed distinctive proton resonances in the aromatic region (δ 7.0–8.5 ppm) along with additional peaks corresponding to heterocyclic or methine protons (δ 4.0–5.5 ppm) and aliphatic protons in the upfield region. Finally, the molecular weight of the compound D4 was determined on the basis of ESI–MS analysis and a peak in m/z 305.3007 correspond

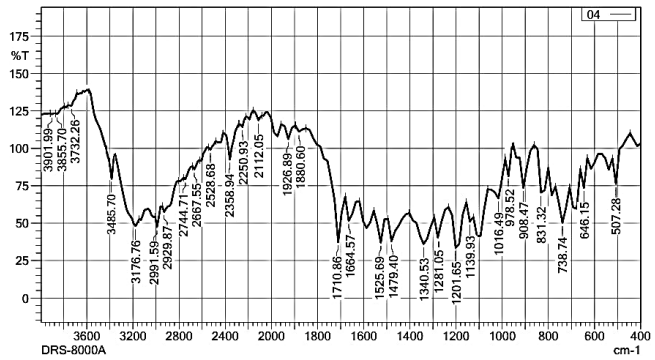


Figure 3: *FTIR spectrum of synthesized compound O4 depicting characteristic absorption bands observed in the range of 4000–400 cm⁻¹, confirming the presence of important functional groups associated with the synthesized tetrahydropyrimidine derivative.*

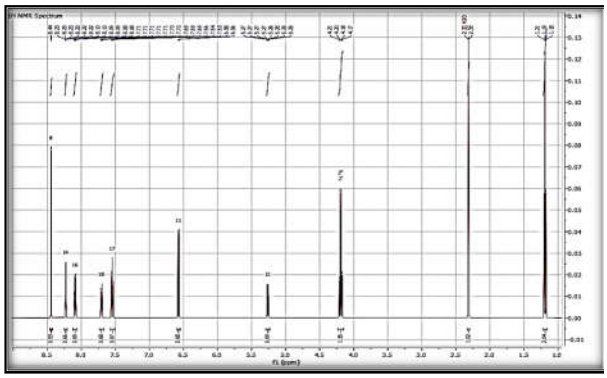


Figure 4: *¹H NMR spectrum of synthesized compound O4 showing characteristic chemical shifts and proton environments corresponding to aromatic ring protons, heterocyclic moieties, and substituted functional groups, confirming the proposed molecular structure.*

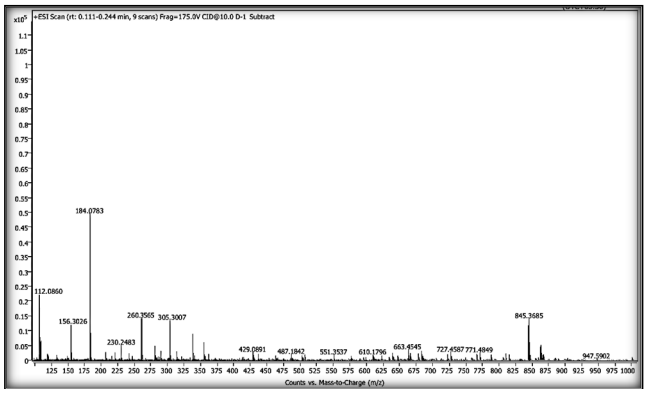


Figure 5: *Mass Spectrum of synthesized compound O4 illustrating the observed mass-to-charge (m/z) values and fragmentation pattern, confirming the molecular mass and supporting the structural elucidation of the synthesized derivative.*

Biological Evaluation

Antimicrobial Activity

All the synthesized derivatives were tested for antimicrobial activity. The MIC of derivativesis found between 4mg/ml to 8mg/ml.

Table 8: *Minimum inhibition concentration (MIC) of synthesized derivatives*

Derivatives	Microorganism			
	Bacteria			Fungi
	S. aureus	B. subtilis	E. coli	C. albicans
D1	5mg/ml	5mg/ml	4mg/ml	6mg/ml

D2	4mg/ml	6mg/ml	7mg/ml	6mg/ml
D3	5mg/ml	6mg/ml	6mg/ml	7mg/ml
D4	6mg/ml	5mg/ml	5mg/ml	5mg/ml
D5	7mg/ml	6mg/ml	6mg/ml	6mg/ml
D6	6mg/ml	6mg/ml	6mg/ml	5mg/ml

(Minimum inhibitory concentration is the smallest amount of the antibacterial substance that will inhibit any further growth of the microbe. Minimum Inhibitory Concentration of all the derivatives tested on the microorganism selected.)

Table 9: *Antibacterial Activity of synthesized derivatives (zone of inhibition in mm)*

Derivatives	**Zone of Inhibition (mm)**		
	Gram positive (+ve)		**Gram negative (-ve)**
	S. aureus	**B. subtilis**	**E.coli**
Norfloxacin	27.50 ± 1.29	21.75 ± 1.70	25.00 ± 2.16
D1	14.00 ± 1.82	12.00 ± 0.81	16.75 ± 0.50
D2	14.25 ± 1.70	15.00 ± 0.50	15.00 ± 2.16
D3	18.00 ± 1.63	15.00 ± 2.16	25.00 ± 0.81
D4	18.25 ± 1.50	18.25 ± 0.95	16.00 ± 0.81
D5	12.25 ± 0.95	13.75 ± 0.95	13.50 ± 0.57
D6	14.25 ± 1.95	12.00 ± 0.95	14.50 ± 1.95

{**Note:** All the data was collected and statistically compared with data of antibacterial agent Norfloxacin. Data are expressed as a zone of inhibition in terms of mean ± S.D. These values were analyzed using graph pad Prism software followed by two-way ANOVA and ***p ≤ 0.0001 was considered statistically significant.}

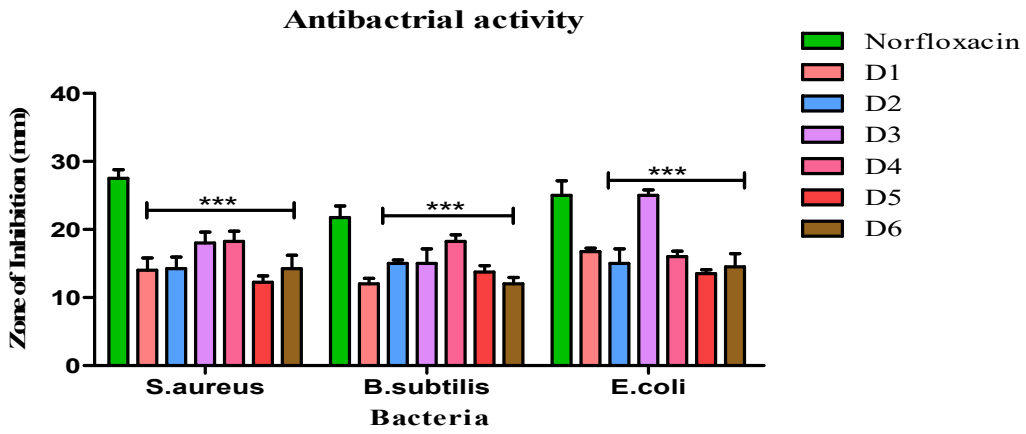


Figure 6: *Comparative evaluation of antibacterial activity of synthesized compounds D1–D6 and standard Norfloxacin against Gram-positive and Gram-negative bacterial strains. Results are represented as zone of inhibition (mm), indicating the relative antimicrobial potency of the synthesized derivatives.*

The above graph showed that the all derivatives showed significant result for inhibition as compared to norfloxacin against gram-positive and gram-negative bacteria. i.eS.aureus,B subtilis, E. coli.

The derivative D3 and D4 showed ***p<0.001 significant results for inhibitions against the gram-positive bacteria S. aureus as compared to norfloxacin.

In case of B. subtilis, D4 showed ***p<0.001 significant result for inhibition as compared to norfloxacin.

In case of E coli derivative D3 showed ***p<0.001 significant results for inhibition as compared to norfloxacin.

The synthesized derivatives showed antibacterial activity against s. aureus, B. subtilis and E. coli

Table 10: *Antifungal Activity of synthesized derivatives (zone of inhibition in mm)*

Derivatives	**Zone of inhibition (mm)**
	C.albicans
Miconazole	35.75 ± 1.50
D1	15.25 ± 1.50
D2	14.00 ± 2.94
D3	26.00 ± 2.44
D4	27.75 ± 2.03
D5	20.00 ± 1.82
D6	19.50 ± 1.00

(Note: All the data was collected and statistically compared with the antifungal agent Miconazole. Data are expressed as a zone of inhibition in terms of mean ± S.D. These values were analyzed using graph pad Prism software followed by one-way ANOVA and ***p < 0.0001 was considered statistically significant.)

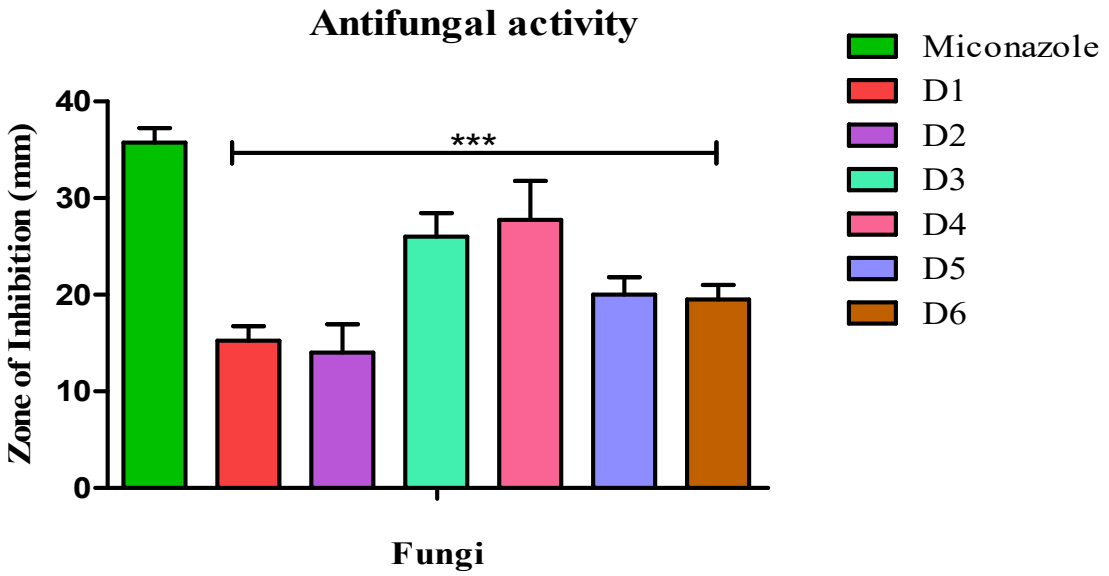


Figure 7: *Comparative evaluation of antifungal activity of synthesized compounds D1–D6 and standard Miconazole against* Candida albicans*. Results are represented as zone of inhibition (mm), indicating the relative antifungal potency of the synthesized derivatives.*

The above graph showed that the all derivatives showed significant results for inhibition against C. albicans as compared to standard miconazole. That means it shows good activity against C. albicans.

In the case of Derivative D1-D6, showed ***p<0.0001 significant result for inhibition against C. albicans as compared to miconazole.

The Derivatives D3 and D4 showed good activity ***p<0.0001 significants result for inhibition against C. albicans.

The synthesis derivatives showed antifungal activity against C. albicans.

CONCLUSION

In this current investigation, the successful design, synthesis, characterization, and biological assessment of novel 1,2,3,4-tetrahydropyrimidine analogues prepared using a microwave-assisted strategy have been achieved. As compared to the conventional approach, the microwave-assisted method yielded a more efficient reaction process, lessened reaction time, increased sustainability, and good yields (70–80%). A total of 24 analogues have been designed utilizing computational approaches and then prepared using the one-pot multicomponent reaction strategy followed by the physicochemical and spectroscopic characterization, i.e., UV-Vis, FTIR, ^{1}H-NMR, and MS, which confirmed their intact structure.

Pharmacokinetics evaluation and drug-likeness analysis of the synthesized analogues suggested good ADME behavior and low toxicity profile, which made them a good candidate for drug discovery. The molecular docking analysis against the chosen bacteria and fungal protein targets (4URM, 6A4M, 2FV5, and 6AKZ) suggested significant binding efficacy for the tested analogues D1–D6 that were similar or superior to the benchmark drug molecules.

The biological results were consistent with the high level of antimicrobial activity against both Gram-positive bacteria such as Staphylococcus aureus and Bacillus subtilis and Gram-negative bacteria like Escherichia coli and the pathogenic fungus Candida albicans. In this regard, D3 and D4 compounds revealed the highest levels of antimicrobial activity against bacteria and fungi with the lowest MIC values and statistically significant inhibition zones. Structural analysis via FTIR and ^{1}H NMR spectrum was also performed on the D4 compound to confirm its molecular structure based on mass spectral results.

To sum up, tetrahydropyrimidine derivatives D3 and D4 are considered good lead molecules for development into novel antimicrobial agents.

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