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Synthesis, Characterization, Antimicrobial And In-Silico Predictions Of 5,6-Dihydropyrimidin-2(1H)-One And 4,5-Dihydro-1H-Pyrazole Derivatives

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ABSTRACT

Two new chalcone-based derivatives produced using a simple condensation technique with starting compound 1 with phenylhydrazine (2) and urea (4) derivatives 3 and 5 are presented in the current work together with their design, synthesis, and biological evaluation. FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS tests were employed to verify the structure. Against four bacterial strains Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosa and two fungal strains Trichoderma asperellum and Candida parapsilosis the synthesized chemicals were tested for antimicrobial activity. Derivative 5 showed significant antibacterial activity, having a 14 mm zone of inhibition against Staphylococcus aureus, 8 mm against E. coli, and 9 mm against P. aeruginosa, with no inhibition against B. subtilis. In antifungal tests at 1000 µM concentration, it showed inhibition zones of 9. 33 mm against T. asperellum and 9. 33 mm against C. parapsilosis. Derivative 3 showed mild activity with maximum inhibition zones of 8 mm (S. aureus, E. coli, P. aeruginosa) and 9. 66 mm (T. asperellum, C. parapsilosis) in comparison. In-silico ADMET profiling of both compounds, done with AdmetLab 3.0 and SwissADME instruments, revealed acceptable absorption, distribution, metabolism, excretion, and toxicity profiles similar to those of the standard drugs Amphotericin B and Ciprofloxacin. These findings show the potential of chalcone-based templates as possible antibacterial agents.

Keywords: Antibacterial activity; Antimicrobial activity; Zone of Inhibition; Synthesis; in-silico ADMET profiling

1. INTRODUCTION

Heterocyclic compounds, also called heterocycles, are organic compounds with a ring structure containing one or more heteroatoms [1]. Heterocycles can be cyclic or acyclic. The general structure of heterocycles is similar to simple organic compounds with only one carbon atom, but the replacement of one or more carbon atoms by heteroatoms gives heterocycles physical properties that differ from analogs all carbon rings. Heterocycles have many applications, including agriculture, medicine, and veterinary medicine [2-5]. These compounds are also used in pesticides, antioxidants, copolymers, corrosion inhibitors, paints and other applications.

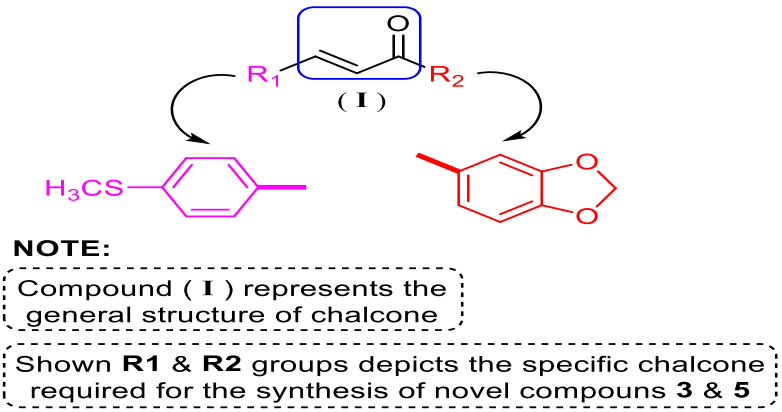


Figure-1: General Structure of Chalcone

Heterocycles are used to produce a variety of chemical compounds. Heterocyclic compounds are found in a variety of substances, mostly natural, including alkaloids, morphine, vinblastine and reserpine, and antibiotics such as cephalosporins and penicillin [6]. According to statistics, more than 85% of all organic compounds form heterocycles. This shows the importance of heterocycles in the design of modern drugs. All heterocycles, synthetic and natural, show pharmacological effects. Heterocyclic molecules, which have physiological and pharmacological activities, have attracted the attention of medical research [7-8]. Many biochemicals found in living organisms, including vitamins, hormones, and antibiotics, are made of heterocyclic molecules [9].

Heterocyclic compounds with nitrogen atoms in the structure are the main class of substances among the active complexes of the body, natural products and common substances in medicinal chemistry [10]. Quinolines, indoles, pyrroles, and pyrrolidines are nitrogen-containing heterocyclic compounds that have become popular in various fields of study, including chemical synthesis and medicine [11]. Due to various applications, the formation of heterocyclic compounds has become a focus in organic synthesis. Many systematic approaches for manufacturing nitrogen-containing heterocyclic compounds were proposed and established in the previous decades [12].

In addition to large-scale study into heterocycles, notably nitrogen heteroatom-based heterocycles, scientists have shown a strong interest in other heterocycles, such as sulfur-containing heterocyclic compounds. Sulfur-containing heterocyclic compounds account for a significant share of FDA-approved medications and therapeutically dynamic structures [13]. These compounds have been shown to have anti-diabetic [14], antibacterial [15-16], anticancer [17-19], antiviral [20], antimicrobial [21-22], anti-inflammatory [23], antihypertensive [24], antimalarial [25-26], anti-Alzheimer's [27-28], antifungal [29-30], and other biological effects. Sulfur-containing heterocyclic compounds are commonly used in chemical research and are found in a wide range of natural products and medications [31]. In addition, sulfur-containing heterocyclic compounds are used to flavor a variety of foods, including meat, vegetables, peanuts, coffee, and chocolate. Several FDA-approved drugs are sulfur heterocycles, including clopidogrel, raloxifene, and rosiglitazone, which treat obesity, breast cancer, and diabetes, respectively [32]. Similarly, ritonavir is a well-known antiviral drug. Thiabendazole can act as an antifungal agent. In addition, many drugs containing sulfur heterocycles have been approved by the FDA and are used to treat a wide range of diseases.

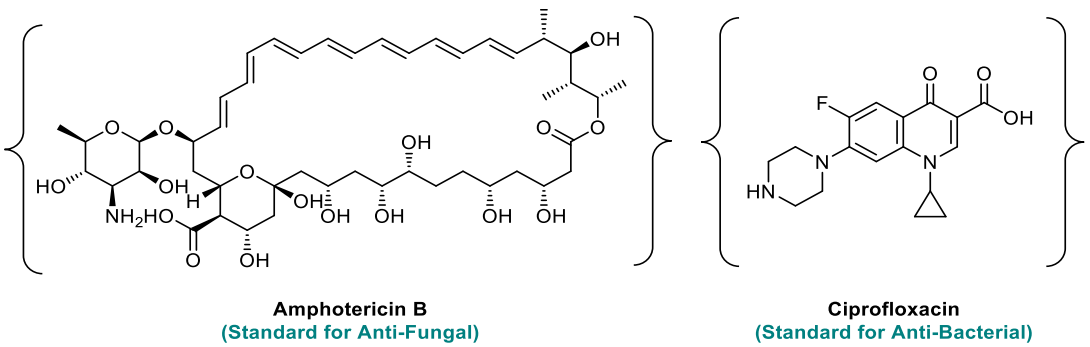


Figure-2: Chemical Structures for Amphotericin B and Ciprofloxacin

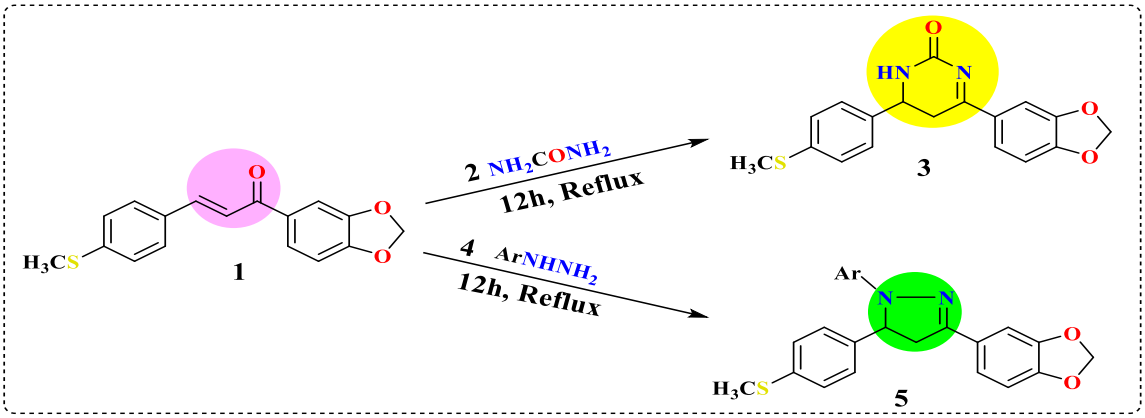
2. MATERIAL AND METHODS

2.1 Synthesis

All the chemicals and solvents used in the synthesis were purchased by the SigmaAldrich and were not purified before the analysis. Various analytical techniques like FTIR, ^{1}H NMR, ^{13}C NMR and HRMS were used for confirmation of the synthesized derivatives. Thin Layer Chromatography were also performed in order the confirm the synthesis before analytical check-ups. The derivatives were also purified by the Column Chromatography in order to yield a better and purified version of the synthesized derivative.

2.2 General Procedure

0.5 mmol. of **1** was taken and made it to react with urea (2) / phenyl hydrazine (**4**) for about 12hrs on the reflux condition at about 50°C. After this period of time a precipitate was obtained in the rb flask which was then filtered and collected. The precipitate thus obtained was the novel derivative of **1** in association with urea.



Scheme-1: Synhesis of the 5,6-dihydropyrimidin-2(1H)-one (3) and 4,5-dihydro-1H-pyrazole (5)

2.3 Anti-Microbial Evaluation

2.3.1 Staphylococcus aureus

By the Zone Inhibition Method (Kirby-Bauer method), the antibacterial activity was verified. Spreading with 100 µl of Bacterial culture Staphylococcus aureus (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL from Mueller- Hinton Broth) and then putting discs bearing 10 µl of various concentration (0 to 100 mM) onto the MHA plates. One disc in each plate was charged with solvent alone, which served as vehicle control; Ciprofloxacin disc (8µg) was taken as positive control. For 24 hours at 37°C, the plates of Staphylococcus aureus were incubated (Basil Scientific Corp. India). Measurements and documenting of the clear regions around the disc were made [33].

2.3.2 Escherichia coli

Following the Zone Inhibition Method (Kirby-Bauer method), the antibacterial action was verified. By distributing 100 µL of bacterial culture, Escherichia coli (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL from Mueller- Hinton Broth) and followed by placing discs holding 10 µl of various concentrations (0 to 100 mM, the MHA plates were inoculated. One disc on each plate served as vehicle control was charged with solvent only; as positive control Ciprofloxacin disc (8µg) was taken. At 37°C for 24 hours, the plates of Escherichia coli were incubated (Basil Scientific Corp. India). Measurements and logging of the distinct zones produced around the disc were made [33].

2.3.3 Bacillus subtilis

Following the Zone Inhibition Method (Kirby- Bauer method), the antibacterial activity was confirmed. Spreading 100 µL of Bacterial culture, Bacillus subtilis (prepared by modifying 0.5 McFarland Unit - Approx. cell density (1.5 X 108 CFU/mL from Mueller-Hinton Broth), the MHA

plates were then treated with discs containing 10 µl of various concentrations (0 to 100 mM). Loading one disc in each plate with solvent only offered vehicle control and taking Ciprofloxacin disc (8 µg) served as positive control. For 24 hours, Bacillus subtilis plates (Basil Scientific Corp. India) were incubated at 37°C. Measurements and recordings of the distinct zones around the disc were done [33].

2.3.4 Pseudomonas aeruginosa

Following the Zone Inhibition Method (Kirby-Bauer method), the antibacterial activity was verified. Spreading with 100 µl of bacterial culture, Pseudomonas aeruginosa, the MHA plates were inoculated; the inoculum was prepared by adjusting 0.5 McFarland Unit - approximately 1.5 X 108 CFU/mL from Mueller- Hinton Broth, and followed by adding 10 µl of varying concentrations (0 to 100 mM). One disc in each plate contained solvent only, acting as vehicle control; a Ciprofloxacin disc (20µg) was taken as positive control. At 37°C for 24 hours, the plates of Pseudomonas aeruginosa were incubated (Basil Scientific Corp. India). Measured and documented were the distinct areas created around the disc [33].

2.3.5 Trichoderma asperellum

Following Zone Inhibition Method (Kirby-Bauer method), the antifungal activity was assessed. Following the inoculation with 100 µl of Fungal culture, Trichoderma asperellum (prepared by modifying 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL from Sabouraud dextrose broth), the SDA plates were followed by placing discs holding 30 µl of varying concentration (0 to 100 mM). One disc on each plate was loaded with solvent solely for vehicle control; 100 µg Amphotericin B disc was adopted as positive control. Trichoderma asperellum plates were incubated for 24 hours at 37°C (Basil Scientific Corp. India- Incubator). Measurements and recording of the clear zones formed around the disc were made [33].

2.3.6 Candida parapsilosis

Following the Zone Inhibition Method (Kirby-Bauer method) confirmed the antifungal action. After setting 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL from Sabouraud dextrose broth, Candida parapsilosis) as the inoculum and followed by setting the discs containing 10 µl of varied concentration (0 to 100 mM), the SDA plates were seeded. One disc in each plate was filled with solvent alone which served as vehicle control; Amphotericin B disc (100 µg) was taken as positive control. C. parapsilosis plates were incubated at 37°C for 24 hours (Basil Scientific Corp. India- Incubator). Measurements of the clear zones around the disc were logged [33].

2.4 ADMET Predictions

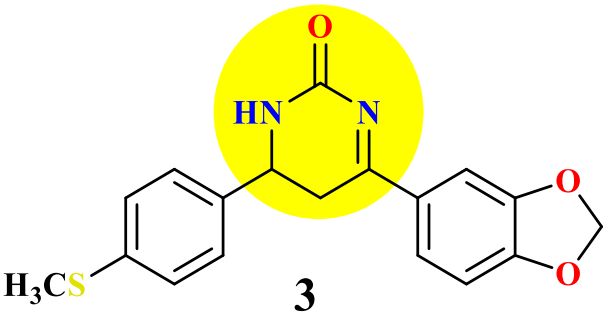
In silico calculations of the synthesized derivatives 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were done by the freely available online web-tools like AdmetLab3.0 and AdmetSAR3.0 [34-57] (https://lmmd.ecust.edu.cn/admetsar3/predict.php).

3. RESULT AND DISCUSSION

3.1 Chemistry

A total of two derivatives were synthesized via a common reactant **1** and differ by 2 and 4 in order to yield the novel derivatives **3** and **5** respectively. Various analytical techniques like FTIR, 1H NMR, 13C NMR and HRMS were used for confirmation of the synthesized derivatives.

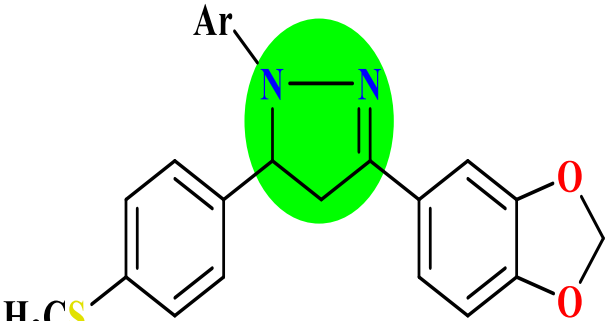
3.1.1 Characterization Data for novel derivative 3



3

Pale yellow solid, yield 76%, mp 115.5 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.38 (3H, s), 2.56 (2H, d, J = 0.768 Hz), 2.81 (1H, d, J = 0.843 Hz), 6.06 (2H, s), 6.98 (1H, d, J = 2.094 Hz), 7.18 (1H, d, J = 2.154 Hz), 7.31 (1H, d, J= 2.193 Hz), 7.40 (1H, s), 7.59 (1H, d, J = 2.277 Hz), 7.92 (1H, d, J = 2.376 Hz); ^{13}C NMR (300 MHz, DMSO-d_6) δ = 14.187, 38.872, 39.083, 39.289, 39.500, 39.711, 39.917, 40.128, 124.738, 125.663, 129.022, 131.116, 141.848, 142.160, 188.301; ES-MS (m/z): 341.09 [M+1]+, calculated for $C_{18}H_{16}N_2O_3S$.

3.1.2 Characterization Data for novel derivative 5



5

Pale yellow solid, yield 79%, mp 121 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.38 (3H, s), 3.69 (2H, d, J = 1.183 Hz), 3.94 (1H, d, J = 1.182 Hz), 6.06 (2H, s), 6.98 (1H, d, J = 2.094 Hz), 7.18 (1H, d, J = 2.154 Hz), 7.31 (1H, d, J= 2.193 Hz), 7.40 (1H, s), 7.59 (1H, d, J = 2.277 Hz), 9.97 (1H, d, J = 2.991 Hz); ^{13}C NMR (300 MHz, DMSO-d_6) δ = 14.935, 38.872, 39.083, 39.289, 39.500, 39.711, 39.917, 40.128, 40.770, 63.222, 101.122, 105.051, 108.179, 119.899, 126.171, 127.268, 127.623, 147.397, 147.560, 148.657; ES-MS (m/z): 313.10 [M+1]+, calculated for $C_{17}H_{16}N_2O_2S$.

3.2 Anti-Bacterial Activity

3.2.1 Staphylococcus aureus

Antibacterial analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Ciprofloxacin against the bacterial strain Staphylococcus aureus. The results obtained from the analysis are given below:

Table-1: Outcomes of the Antibacterial analysis against **Staphylococcus aureus**

Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
Ciprofloxacin (PC)	8µg	29.33
3	50 µM	7
5	50 µM	11

Table-2: Anti-bacterial analysis results of **3** against **Staphylococcus aureus**

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	29	29	29	29	0	0
0	0	0	0	0	0	0
50	7	7	7	7	0	0
125	7	7	7	7	0	0
250	8	8	7	7.666666667	0.5773502692	0.3333333333
500	8	8	8	8	0	0
1000	8	8	8	8	0	0

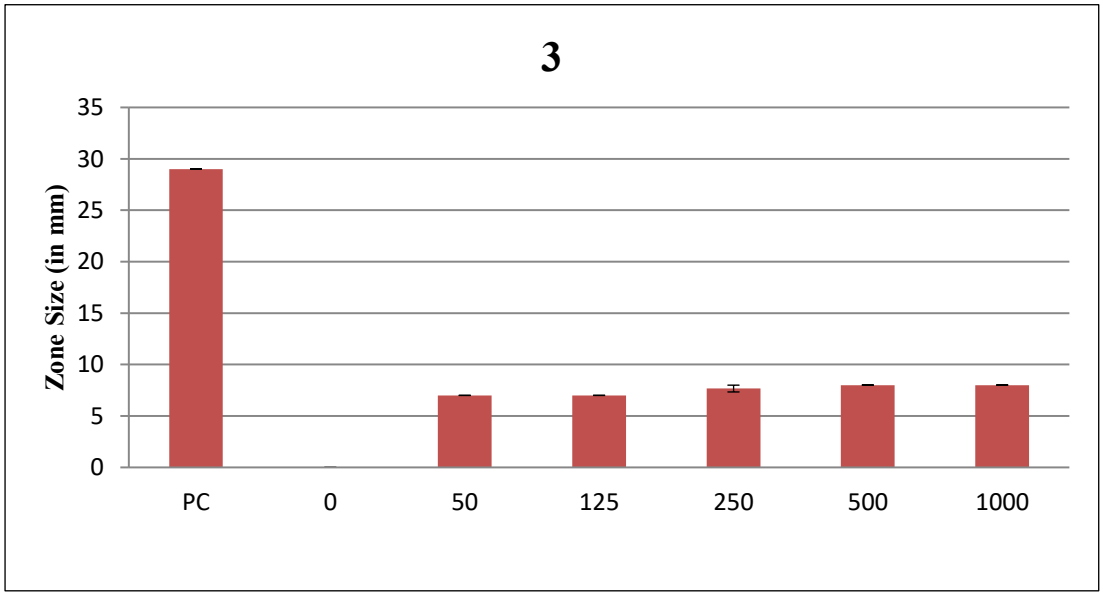


Figure-3: Graphical representation of results of the Antibacterial analysis of derivative **3** against **Staphylococcus aureus**

Table-3: Anti-bacterial analysis results of **5** against **Staphylococcus aureus**

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	28	29	29	28.6666667	0.57735	0.3333
0	0	0	0	0	0	0
50	12	10	11	11	1	0.5774
125	12	12	12	12	0	0
250	12	13	13	12.6666667	0.57735	0.3333
500	14	14	13	13.6666667	0.57735	0.3333
1000	14	14	14	14	0	0

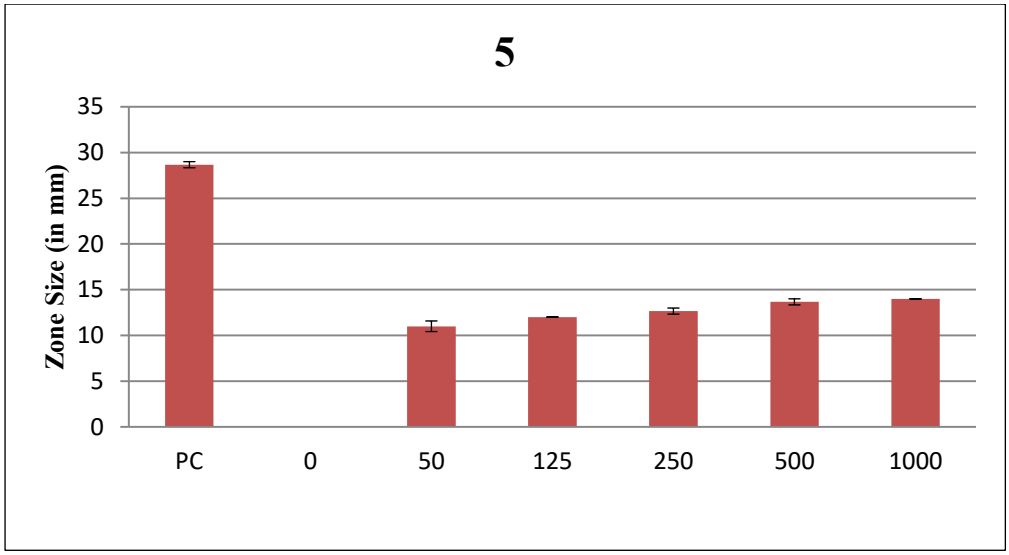


Figure-4: Graphical representation of results of the Antibacterial analysis of derivative **5** against **Staphylococcus aureus**

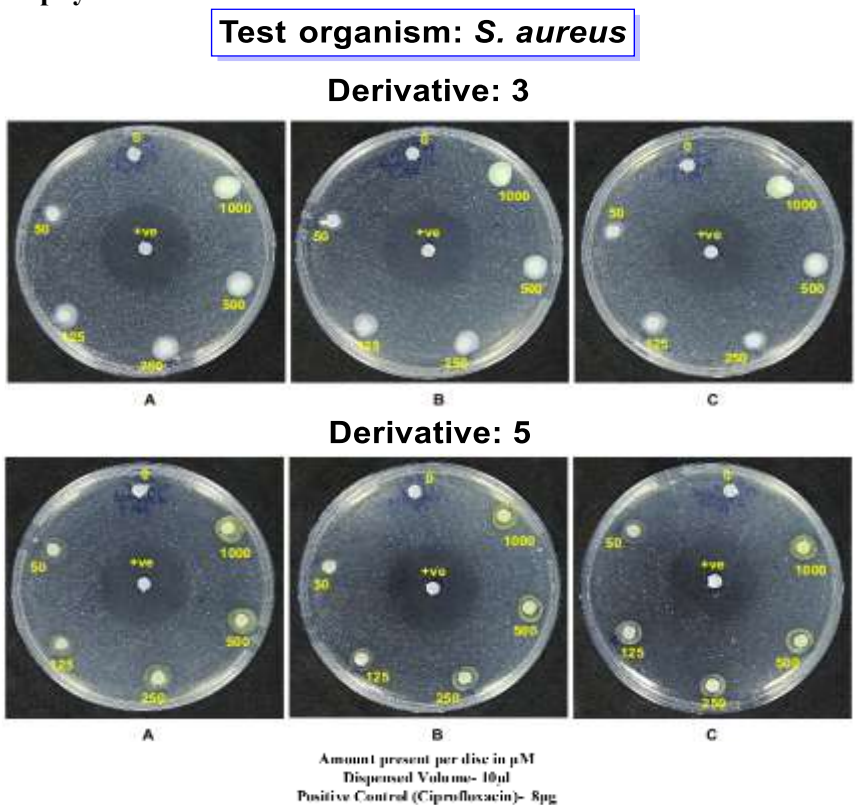


Figure-5: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against **Staphylococcus aureus**

3.2.2 Escherichia coli

Antibacterial analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Ciprofloxacin against the bacterial strain Escherichia coli. The results obtained from the analysis are given below:

Table-4: Outcomes of the Antibacterial analysis against **Escherichia coli**

Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
Ciprofloxacin (PC)	8µg	29.33

3	50µM	6
5	50µM	2.33

Table-5: Anti-bacterial analysis results of **3** against **Escherichia coli**

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	19	19	19	19	0	0
0	0	0	0	0	0	0
50	6	6	6	6	0	0
125	7	7	7	7	0	0
250	7	7	7	7	0	0
500	8	8	8	8	0	0
1000	8	8	8	8	0	0

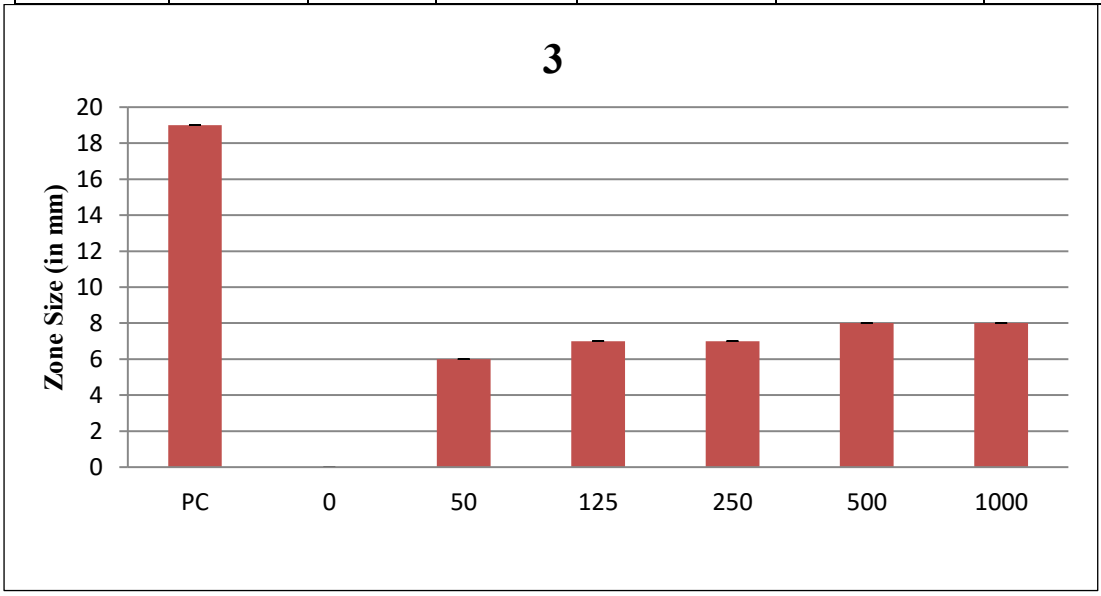


Figure-6: Graphical representation of results of the Antibacterial analysis of derivative **3** against **Escherichia coli**

Table-6: Anti-bacterial analysis results of **5** against **Escherichia coli**

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	19	19	19	19	0	0
0	0	0	0	0	0	0
50	0	0	7	2.33333333	4.04145	2.3333
125	6	7	7	6.66666667	0.57735	0.3333
250	6	7	8	7	1	0.5774
500	8	8	8	8	0	0
1000	8	8	8	8	0	0

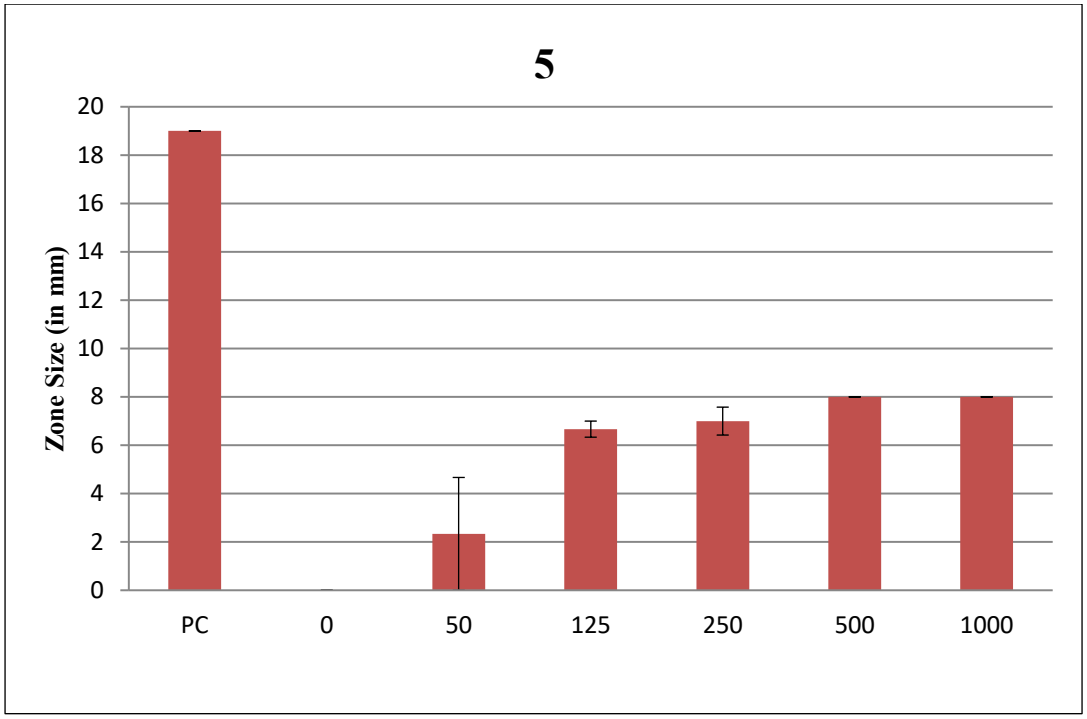


Figure-7: Graphical representation of results of the Antibacterial analysis of derivative **5** against **Escherichia coli**

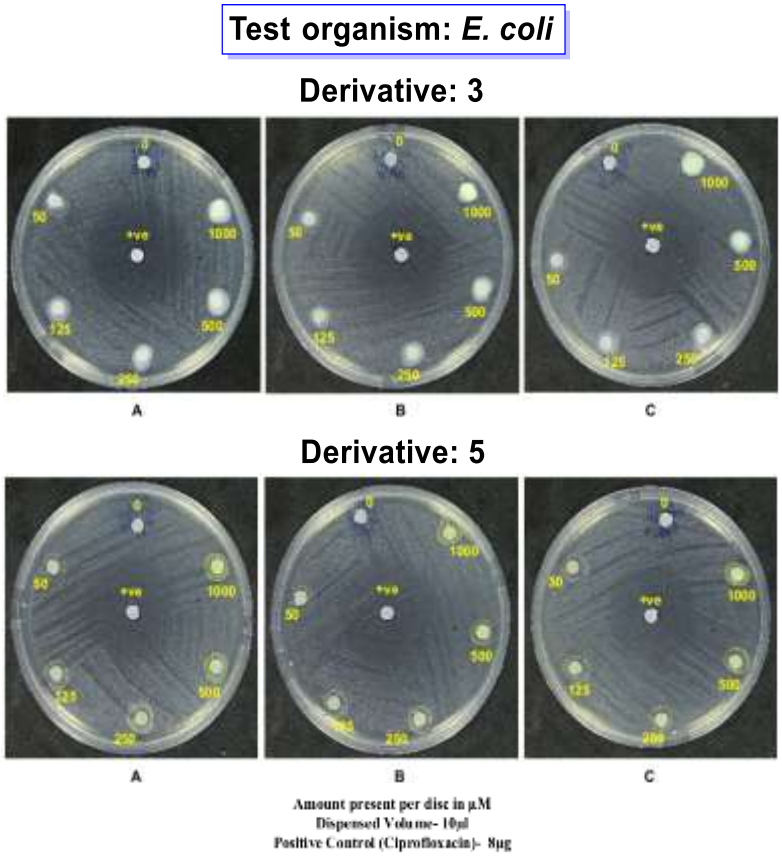


Figure-8: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against **Escherichia coli**

3.2.3 Bacillus subtilis

Antibacterial analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Ciprofloxacin against the bacterial strain **Bacillus subtilis**. The results obtained from the analysis are given below:

Table-7: Outcomes of the Antibacterial analysis against **Bacillus subtilis**

Sample Id	**Effective Amount**	**Average Zone at Effective amount (in mm)**
Ciprofloxacin (PC)	8µg	27
3	50 µM	2
5	-	-

Table-8: Anti-bacterial analysis results of 3 against **Bacillus subtilis**

Amount (µM/disc)	**Plate A**	**Plate B**	**Plate C**	**Average**	**SD**	**SEM**
PC	27	26	26	26.33333333	0.577350269	0.333333333
0	0	0	0	0	0	0
50	6	0	0	2	3.464101615	2
125	6	0	0	2	3.464101615	2
250	6	0	0	2	3.464101615	2
500	6	0	0	2	3.464101615	2
1000	6	6	6	6	0	0

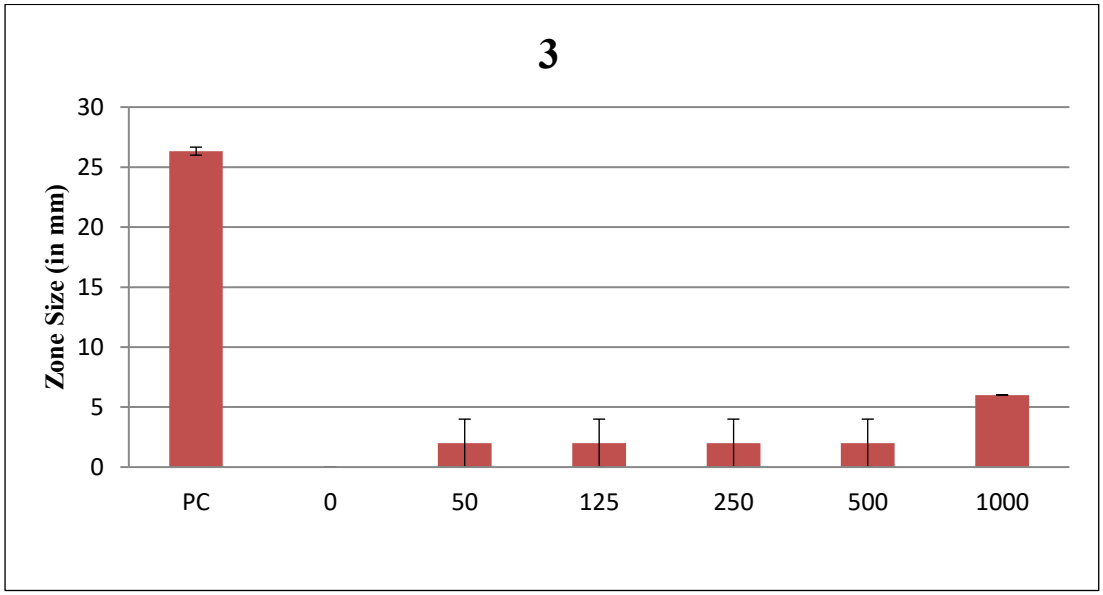


Figure-9: Graphical representation of results of the Antibacterial analysis of derivative **3** against **Bacillus subtilis**

Table-9: Anti-bacterial analysis results of 5 against **Bacillus subtilis**

Amount (µM/disc)	**Plate A**	**Plate B**	**Plate C**	**Average**	**SD**	**SEM**
PC	27	27	27	27	0	0
0	0	0	0	0	0	0
50	0	0	0	0	0	0
125	0	0	0	0	0	0

250	0	0	0	0	0	0
500	0	0	0	0	0	0
1000	0	0	0	0	0	0

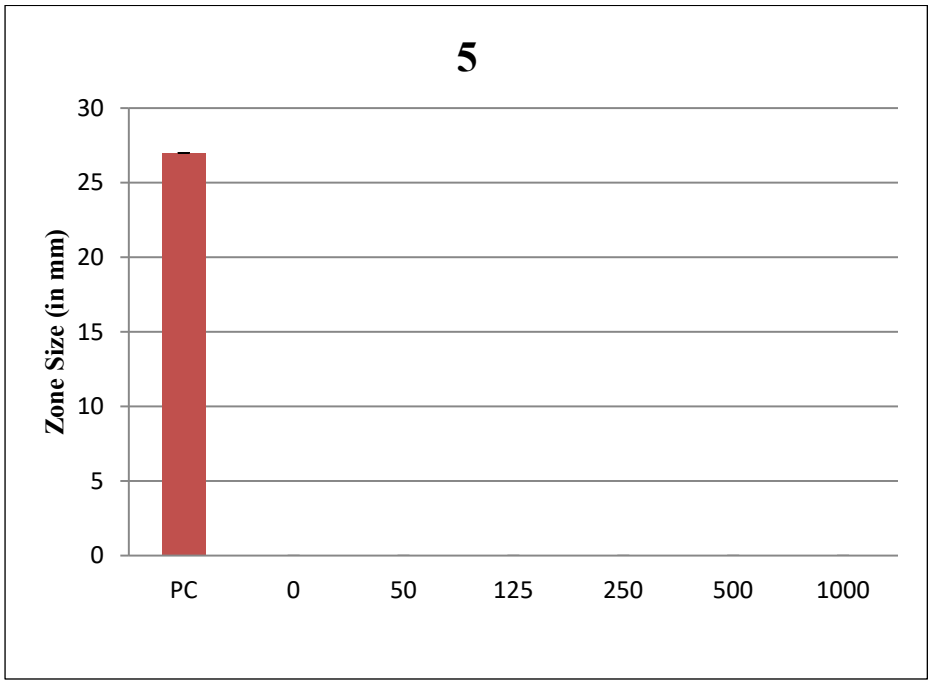


Figure-10: Graphical representation of results of the Antibacterial analysis of derivative **5** against **Bacillus subtilis**

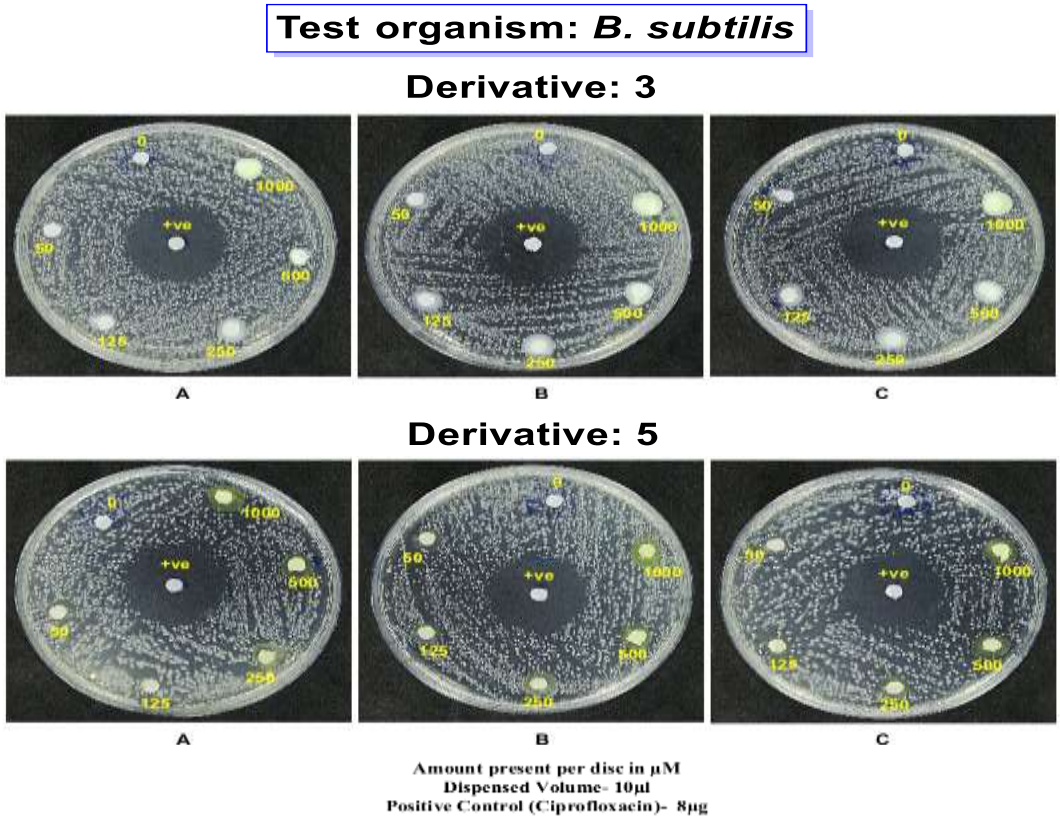


Figure-11: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against B. subtilis

3.2.4 Pseudomonas aeruginosa

Antibacterial analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Ciprofloxacin against the bacterial strain Pseudomonas aeruginosa. The results obtained from the analysis are given below:

Table-10: Outcomes of the Antibacterial analysis against Pseudomonas aeruginosa

Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
Ciprofloxacin (PC)	20µg	28.33
3	50µM	6
5	50µM	6.33

Table-11: Anti-bacterial analysis results of 3 against Pseudomonas aeruginosa

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	28	28	27	27.666667	0.57735	0.33333
0	0	0	0	0	0	0
50	6	6	6	6	0	0
125	6	7	7	6.6666667	0.57735	0.33333
250	7	7	7	7	0	0
500	7	8	7	7.3333333	0.57735	0.33333
1000	8	8	8	8	0	0

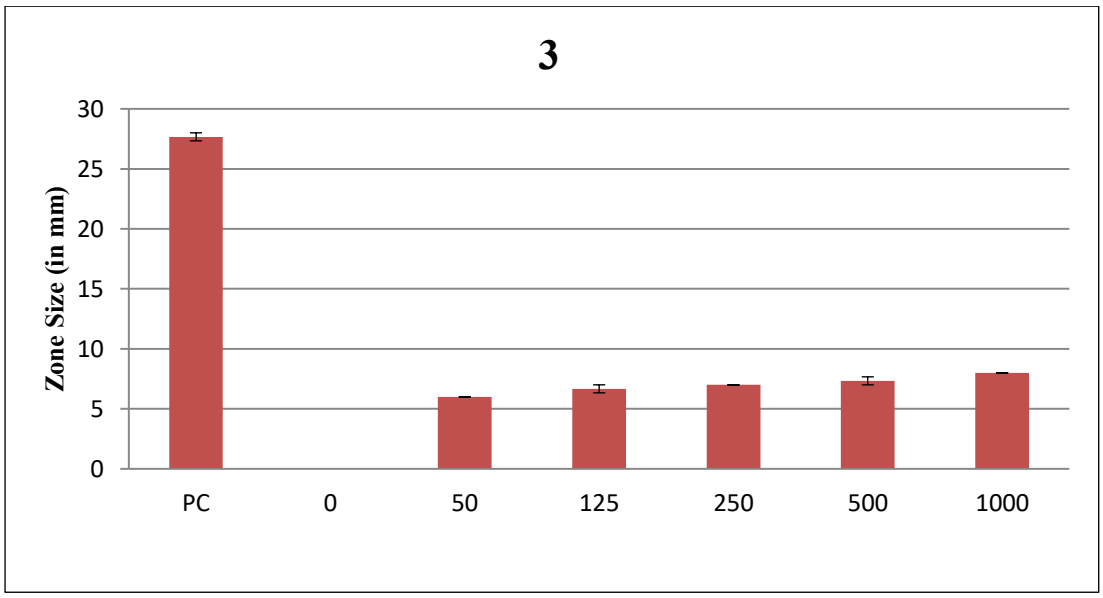


Figure-12: Graphical representation of results of the Antibacterial analysis of derivative **3** against **Pseudomonas aeruginosa**

Table-12: Anti-bacterial analysis results of 5 against Pseudomonas aeruginosa

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	27	26	26	26.3333333	0.57735	0.3333
0	0	0	0	0	0	0
50	6	6	7	6.33333333	0.57735	0.3333

125	7	7	8	7.33333333	0.57735	0.3333
250	8	7	8	7.66666667	0.57735	0.3333
500	8	8	8	8	0	0
1000	9	8	9	8.66666667	0.57735	0.3333

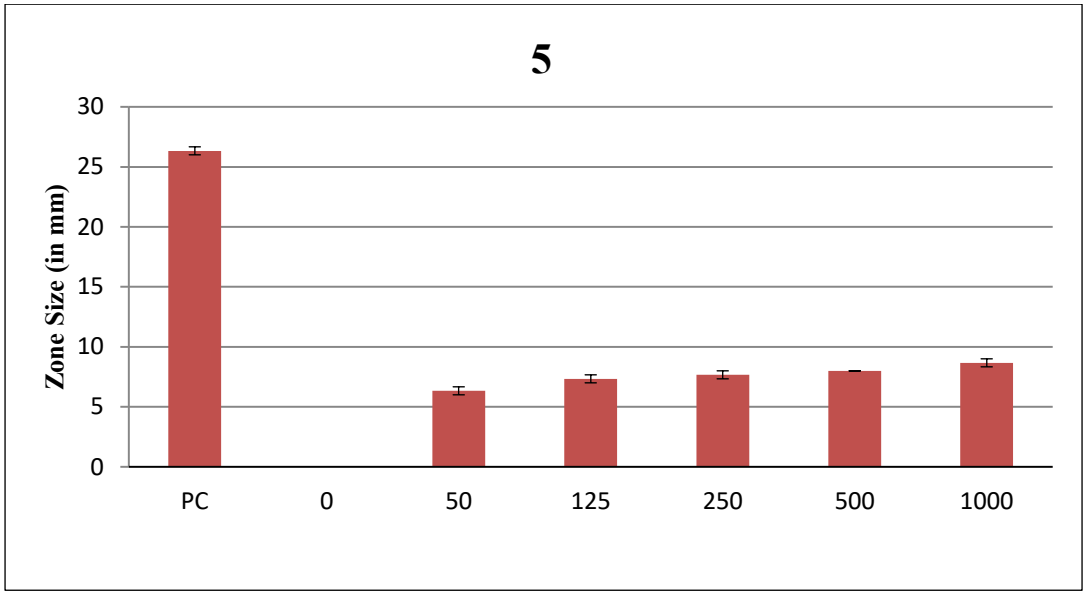


Figure-13: Graphical representation of results of the Antibacterial analysis of derivative **5** against Pseudomonas aeruginosa

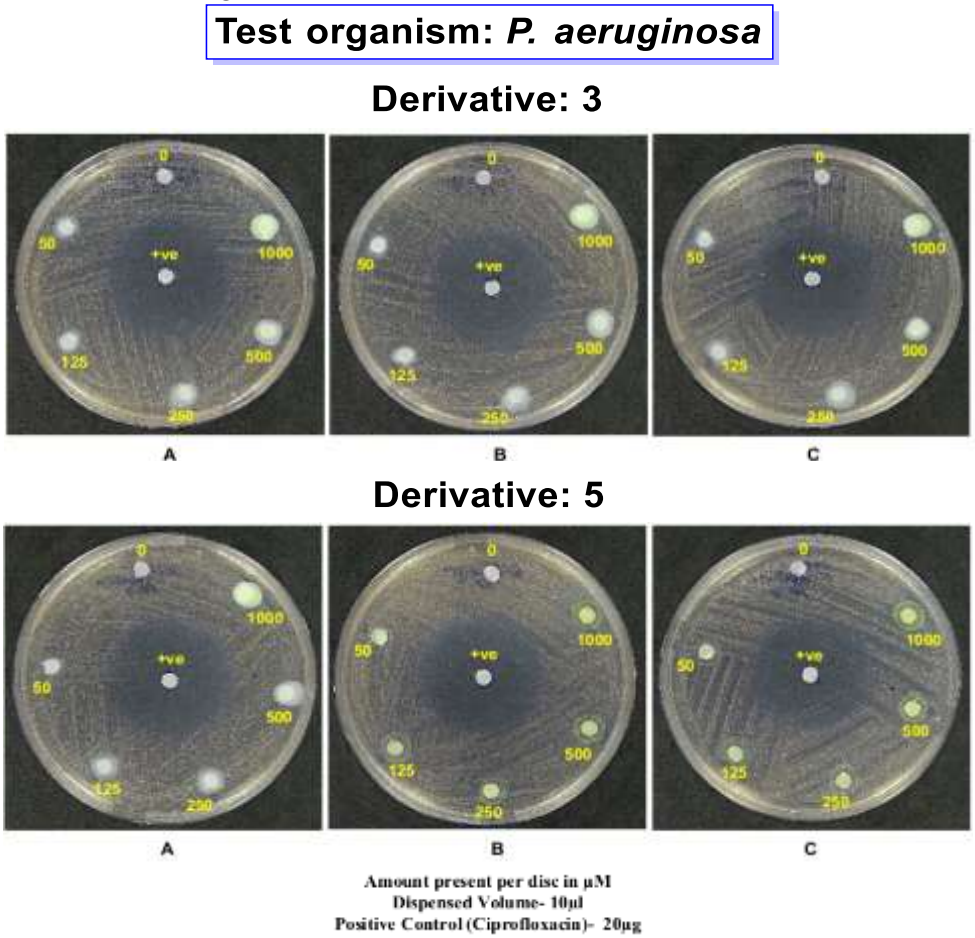


Figure-14: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against Pseudomonas aeruginosa

3.2.5 Trichoderma asperellum

Antifungal analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Amphotericin B against the fungus strain Trichoderma asperellum. The results obtained from the analysis are given below:

Table-13: Outcomes of the Antifungal analysis against Trichoderma asperellum

Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
Amphotericin B (PC)	100µg	17.33
3	50µM	5.33
5	50µM	7.66

Table-14: Anti-fungal analysis results of 3 against Trichoderma asperellum

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	14	15	14	14.333333	0.57735	0.33333
0	0	0		0	0	0
50	0	8	8	5.3333333	4.6188	2.66667
125	10	8	9	9	1	0.57735
250	10	8	9	9	1	0.57735
500	10	8	9	9	1	0.57735
1000	10	9	10	9.6666667	0.57735	0.33333

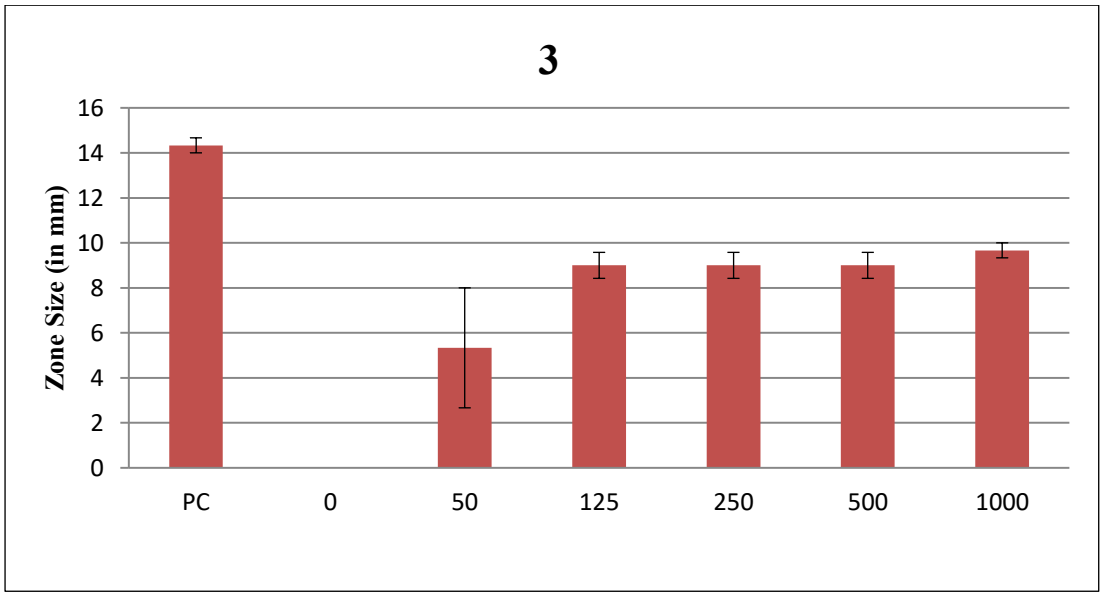


Figure-15: Graphical representation of results of the Antifungal analysis of derivative **3** against Trichoderma asperellum

Table-15: Anti-fungal analysis results of 5 against Trichoderma asperellum

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	14	15	14	14.3333333	0.57735	0.3333
0	0	0	0	0	0	0
50	8	8	7	7.66666667	0.57735	0.3333
125	10	8	8	8.66666667	1.1547	0.6667

250	12	8	8	9.33333333	2.3094	1.3333
500	12	8	8	9.33333333	2.3094	1.3333
1000	12	8	8	9.33333333	2.3094	1.3333

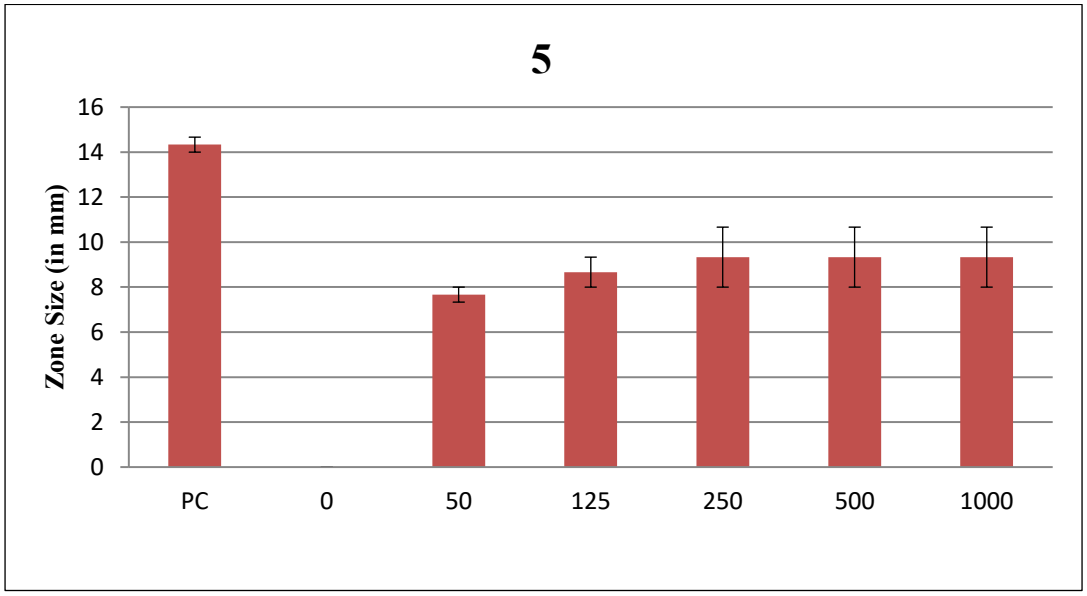


Figure-16: Graphical representation of results of the Antifungal analysis of derivative **5** against Trichoderma asperellum

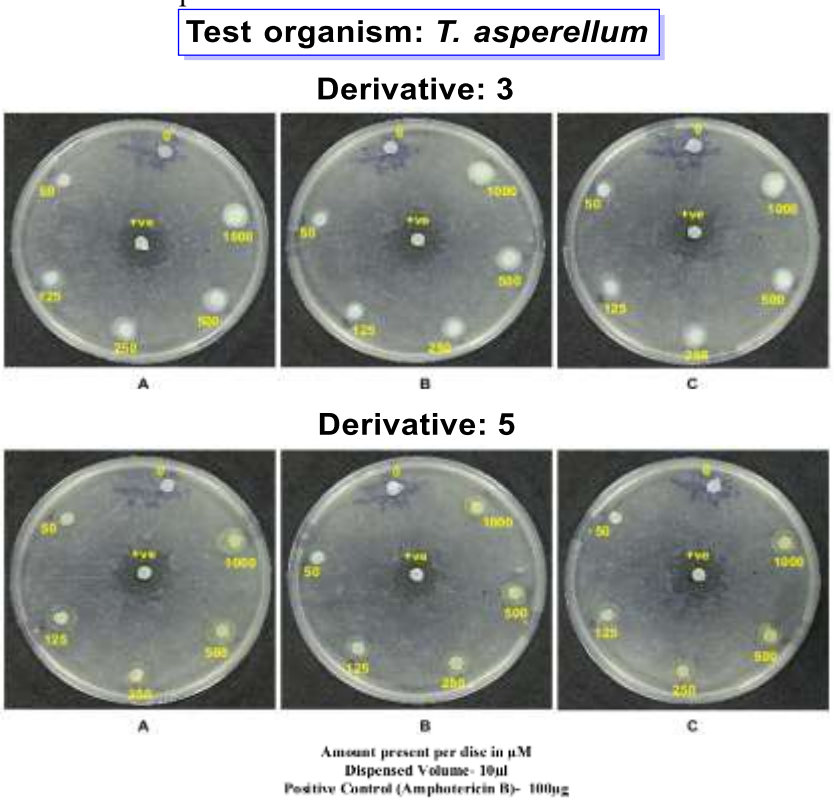


Figure-17: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against Trichoderma asperellum

3.2.6 Candida parapsilosis

Antifungal analysis of the synthesized derivatives 3 & 5 was done and was also compared with the standard drug Amphotericin B against the fungus strain Candida parapsilosis. The results obtained from the analysis are given below:

Table-16: Outcomes of the Antifungal analysis against Candida parapsilosis

Sample Id	Effective Amount	Average Zone at Effective amount (in mm)
Amphotericin B (PC)	100µg	23
3	-	-
5	50µM	6.33

Table-17: Anti-fungal analysis results of 3 against Candida parapsilosis

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	14	15	14	14.333333	0.57735	0.33333
0	0	0		0	0	0
50	0	8	8	5.3333333	4.6188	2.66667
125	10	8	9	9	1	0.57735
250	10	8	9	9	1	0.57735
500	10	8	9	9	1	0.57735
1000	10	9	10	9.6666667	0.57735	0.33333

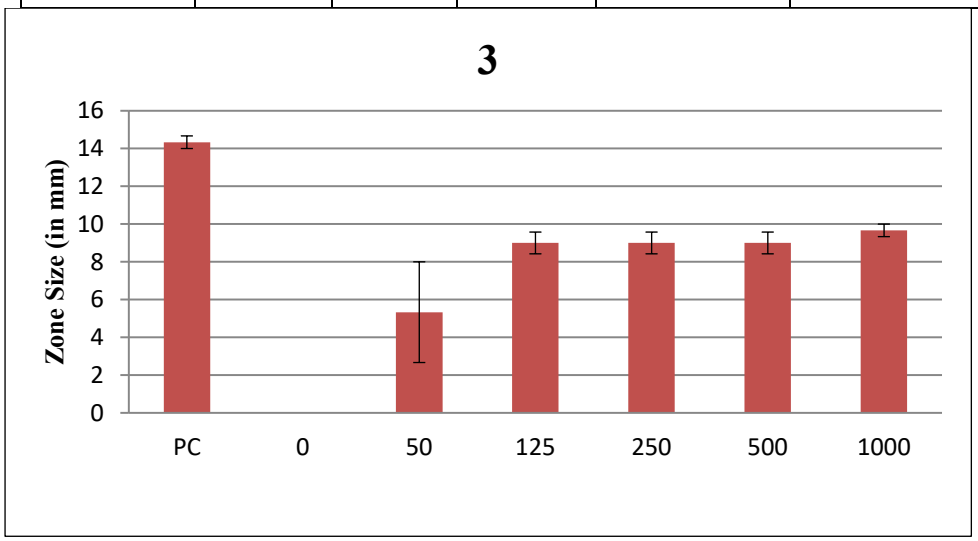


Figure-18: Graphical representation of results of the Antifungal analysis of derivative **3** against Candida parapsilosis

Table-18: Anti-fungal analysis results of 5 against Candida parapsilosis

Amount (µM/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	14	15	14	14.3333333	0.57735	0.3333
0	0	0	0	0	0	0
50	8	8	7	7.66666667	0.57735	0.3333
125	10	8	8	8.66666667	1.1547	0.6667

250	12	8	8	9.33333333	2.3094	1.3333
500	12	8	8	9.33333333	2.3094	1.3333
1000	12	8	8	9.33333333	2.3094	1.3333

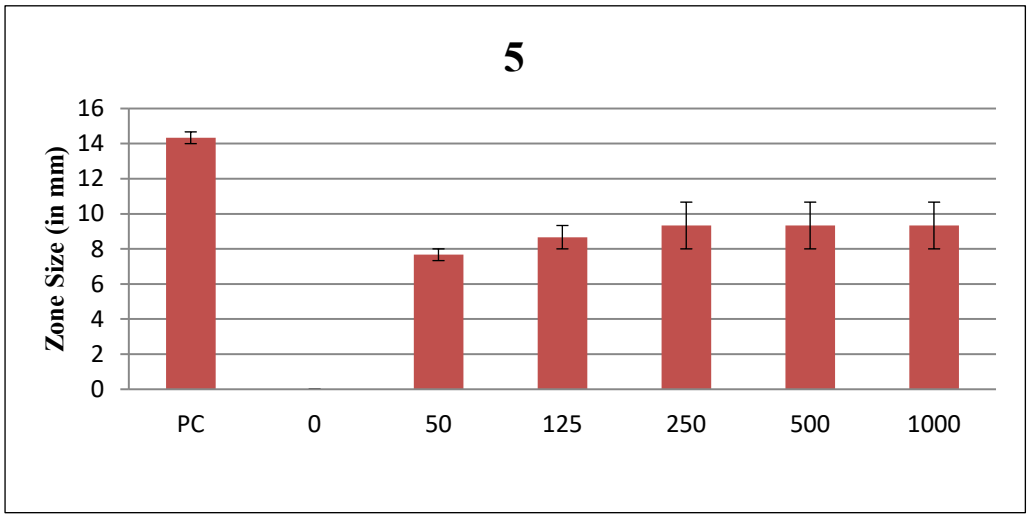


Figure-19: Graphical representation of results of the Antifungal analysis of derivative **5** against Candida parapsilosis

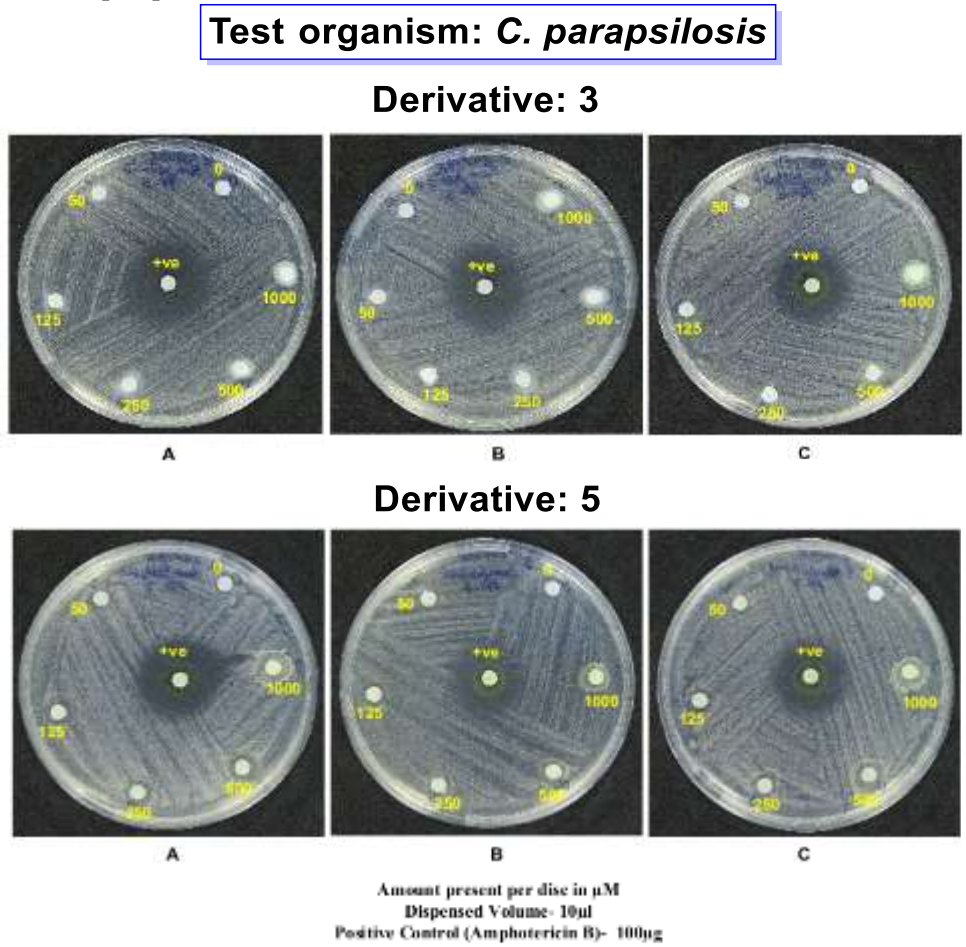


Figure-20: Results of zone inhibition test by Disc Diffusion Method for derivatives 3 and 5 against Candida parapsilosis

3.3ADMET Predictions

3.3.1 Physicochemical Properties

Physicochemical properties of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Molecular Weight, Volume, Density, nHA, nHD, nRot, nRing, MaxRing, nHet, fChar, nRig, Flexibility, Stereo Centers, TPSA, logS, logP, logD7.4, pka (Acid), pka (Base), Melting point and Boiling point.

Table-19: Physico chemical properties of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameters	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
Molecular Weight	340.09	312.09	923.49	331.13
Volume	331.44	307.99	932.501	317.972
Density	1.026	1.013	0.99	1.041
nHA	5.0	4.0	18.0	6.0
nHD	1.0	1.0	13.0	2.0
nRot	3.0	3.0	3.0	3.0
nRing	4.0	4.0	3.0	4.0
MaxRing	9.0	9.0	38.0	10.0
nHet	6.0	5.0	18.0	7.0
fChar	0.0	0.0	0.0	0.0
nRig	23.0	21.0	48.0	22.0
Flexibility	0.13	0.143	0.062	0.136
Stereo Centers	1.0	1.0	19.0	0.0
TPSA	59.92	42.85	319.61	74.57
logS	-3.89	-3.725	-2.696	-1.173
logP	2.643	3.087	0.785	0.096
logD7.4	2.85	3.155	1.309	1.135
pka (Acid)	8.442	10.161	4.656	5.708
pka (Base)	3.72	5.124	6.575	6.911
Melting point	166.548	140.541	167.883	222.611
Boiling point	317.971	313.702	284.045	297.299

3.3.2 Absorption

Absorption properties of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Caco-2 Permeability, MDCK Permeability, PAMPA, Pgp inhibitor, Pgp substrate and HIA were analyed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table-20: Absorption properties of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
Caco-2 Permeability	-5.194	-5.099	-5.715	-5.863
MDCK Permeability	0.0	0.0	0.0	0.0
PAMPA	-	--	+++	+++
Pgp inhibitor	+	++	---	---
Pgp substrate	---	---	+++	+++
HIA	---	---	---	---

3.3.3 Distribution

Distribution properties of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like PPB, VDss, BBB, Fu, OATP1B1 inhibitor, OATP1B3 inhibitor, BCRP inhibitor, MRP1 inhibitor and

BSEP inhibitor were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table-21: Distribution properties of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	3	5	Amphotericin B	Ciprofloxacin
PPB	95.0%	95.0%	89.9%	25.5%
VDss	1.158	2.257	0.459	1.827
BBB	-	+++	---	---
Fu	4.1%	5.1%	11.4%	55.5%
OATP1B1 inhibitor	--	--	++	+++
OATP1B3 inhibitor	-	-	--	+++
BCRP inhibitor	---	-	---	---
MRP1 inhibitor	--	+	+	+++
BSEP inhibitor	+++	+++	---	---

3.3.4 Metabolism

Metabolism properties of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like inhibitor and substrate of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP2B6, CYP2C8 and HLM Stability were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table-22: Metabolism properties of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	3	5	Amphotericin B	Ciprofloxacin
CYP1A2 inhibitor	+++	+++	---	---
CYP1A2 substrate	+++	+++	---	---
CYP2C19 inhibitor	+++	+++	---	---
CYP2C19 substrate	+++	++	---	---
CYP2C9 inhibitor	+	+++	---	---
CYP2C9 substrate	++	-	---	---
CYP2D6 inhibitor	+++	+++	---	---
CYP2D6 substrate	+++	+++	---	---
CYP3A4 inhibitor	+++	+++	---	---
CYP3A4 substrate	---	---	---	---
CYP2B6 inhibitor	+++	+++	---	---
CYP2B6 substrate	-	--	---	---
CYP2C8 inhibitor	---	--	---	---
HLM Stability	++	++	---	---

3.3.5 Medicinal Chemistry

Metabolism properties of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like QED, SAscore, GASA, Fsp^3, MCE-18, NPscore, Lipinski Rule, Pfizer Rule, GSK Rule, GoldenTriangle, PAINS, Alarm_NMR Rule, BMS Rule, Chelating Rule, Colloidal aggregators, FLuc inhibitors, Blue fluorescence and Green fluorescence were analysed.

Table-23: Medicinal chemistry properties of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	3	5	Amphotericin B	Ciprofloxacin
QED	0.864	0.88	0.174	0.893
SAscore	Easy	Easy	Hard	Easy
GASA	Easy	Easy	Hard	Easy
Fsp^3	0.222	0.235	0.66	0.412
MCE-18	65.455	62.429	86.154	56.833

NPscore	-0.355	-0.664	1.751	-0.725
Lipinski Rule	Accepted	Accepted	Rejected	Accepted
Pfizer Rule	Accepted	Rejected	Accepted	Accepted
GSK Rule	Accepted	Accepted	Rejected	Accepted
GoldenTriangle	Accepted	Accepted	Rejected	Accepted
PAINS	0	0	0	0
Alarm_NMR Rule	3	1	1	1
BMS Rule	0	0	1	0
Chelating Rule	0	0	0	0
Colloidal aggregators	0.937	0.996	0.675	0.182
FLuc inhibitors	0.798	0.997	0.507	0.08
Blue fluorescence	0.107	0.062	0.105	0.778
Green fluorescence	0.592	0.854	0.201	0.164
Reactive compounds	0.032	0.026	0.344	0.003
Promiscuous compounds	0.002	0.113	0.347	0.395

3.3.6 Toxicophore Rules

Toxicophore rules of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Aquatic Toxicity Rule, Genotoxic Carcinogenicity Mutagenicity Rule, NonGenotoxic Carcinogenicity Rule, Skin Sensitization Rule, Acute Toxicity Rule, NonBiodegradable, SureChEMBL Rule and FAF-Drugs4 Rule were analysed.

Table-24: Toxicophore rules of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
Aquatic Toxicity Rule	0	0	1	1
Genotoxic Carcinogenicity Mutagenicity Rule	0	0	0	1
NonGenotoxic Carcinogenicity Rule	0	0	0	1
Skin Sensitization Rule	2	1	2	0
Acute Toxicity Rule	0	0	0	1
NonBiodegradable	0	0	1	2
SureChEMBL Rule	0	0	1	0
FAF-Drugs4 Rule	1	1	0	1

3.3.7 Toxicity

Toxicity parameters of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like hERG Blockers, Carcinogenicity, hERG Blockers (10 um), Skin Sensitization, DILI, AMES Toxicity, Rat Oral Acute Toxicity, FDAMDD, Eye Corrosion, Eye Irritation, Drug-induced Nephrotoxicity, Respiratory, Human Hepatotoxicity, Drug-induced Neurotoxicity, Ototoxicity, Hematotoxicity, Genotoxicity, RPMI-8226 Immunitoxicity, RPMI-8226 Immunitoxicity, A549 Cytotoxicity, Hek293 Cytotoxicity, BCF, IGC50, LC50DM and LC50FM were analysed.

Table-25: Toxicity details of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
hERG Blockers	0.187	0.326	0.0	0.466
hERG Blockers (10um)	0.658	0.715	0.0	0.105
DILI	1.0	0.999	0.064	0.996
AMES Toxicity	0.51	0.377	0.252	0.761
Rat Oral Acute Toxicity	0.423	0.592	0.093	0.743
FDAMDD	0.512	0.539	0.0	0.481

Skin Sensitization	0.324	0.271	1.0	0.166
Carcinogenicity	0.923	0.872	0.002	0.191
Eye Corrosion	0.0	0.0	0.931	0.0
Eye Irritation	0.054	0.137	0.978	0.215
Respiratory	0.797	0.873	0.0	0.99
Human Hepatotoxicity	0.717	0.598	0.001	0.983
Drug-induced Nephrotoxicity	0.772	0.726	1.0	0.999
Drug-induced Neurotoxicity	0.951	0.897	0.0	0.996
Ototoxicity	0.739	0.718	1.0	0.982
Hematotoxicity	0.764	0.719	0.186	0.869
Genotoxicity	0.999	0.984	0.0	1.0
RPMI-8226 Immunitoxicity	0.083	0.145	0.155	0.177
A549 Cytotoxicity	0.083	0.315	0.598	0.088
Hek293 Cytotoxicity	0.657	0.732	0.0	0.052
BCF	1.267	1.982	0.186	0.161
IGC50	3.737	3.907	2.453	2.938
LC50DM	5.33	5.664	4.236	4.359
LC50FM	4.697	4.916	3.19	3.535

3.3.8 TOX21 Pathway

Tox21 Pathway parameters of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetLab3.0. Parameters like NR-AhR, NR-AR, NR-AR-LBD, NR-Aromatase, NR-ER, NR-ER-LBD, NR-PPAR-gamma, SR-ARE, SR-ATAD5, SR-HSE, SR-MMP and SR-p53 were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table-26: TOX21 details of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
NR-AhR	++	+++	---	---
NR-AR	---	---	---	--
NR-AR-LBD	---	-	---	---
NR-Aromatase	---	--	---	---
NR-ER	+	++	---	+
NR-ER-LBD	---	---	---	---
NR-PPAR-gamma	---	---	---	---
SR-ARE	-	-	+++	---
SR-ATAD5	---	--	---	---
SR-HSE	---	++	---	---
SR-MMP	-	++	---	---
SR-p53	-	+++	---	---

3.3.9 Radar View

Radar View of the synthesized 3 and 5 along with the standards like Amphotericin B and Ciprofloxacin were generated by the freely available online web-tool AdmetLab3.0.

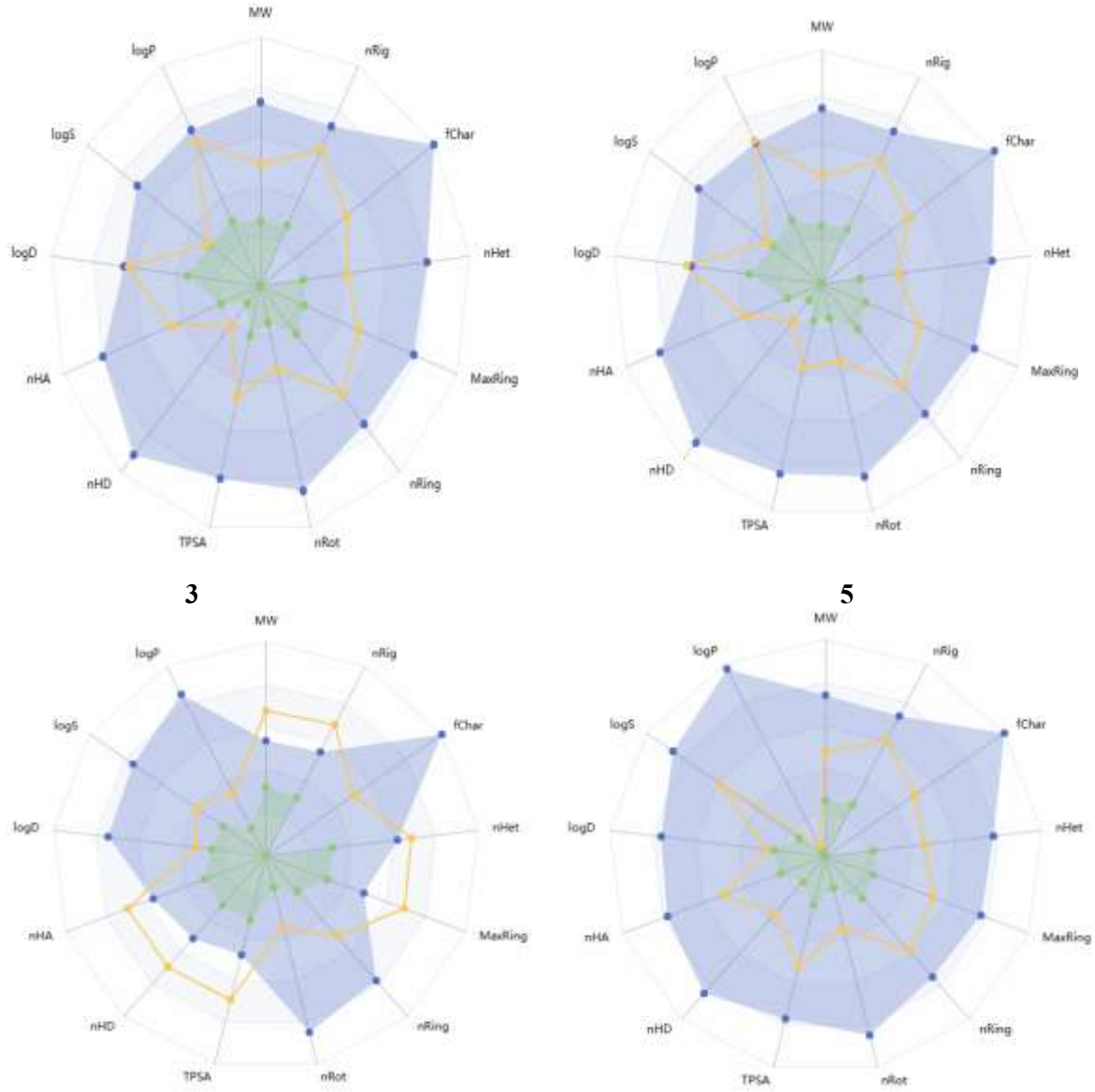


Figure-21: Radar View representation of 3 and 5 along with Amphotericin B and Ciprofloxacin

3.3.10 Cosmetic Risk Assessment

Cosmetic Risk Assessment parameters of the synthesized 3 and 5 along with the standard drugs like Amphotericin B and Ciprofloxacin were calculated by the freely available online web-tool AdmetSAR3.0. Parameters like Eye corrosion, Eye irritation, Skin corrosion, Skin irritation, Skin sensitisation, Acute dermal toxicity, Photoinduced toxicity, Phototoxicity and Photoallergy. https://lmmd.ecust.edu.cn/admetsar3/predict.php (web-link).

Table-27: Cosmetic risk assessment of 3 and 5 along with Amphotericin B and Ciprofloxacin

Parameter	**3**	**5**	**Amphotericin B**	**Ciprofloxacin**
Eye corrosion	0	0	0	0
Eye irritation	0	0	0	0
Skin corrosion	0	0	0	0
Skin irritation	0	0	0	0
Skin sensitisation	0	0	0	0

Acute dermal toxicity	1	1	1	1
Photoinduced toxicity	0	0	0	1
Phototoxicity	0	0	0	1
Photoallergy	0	0	0	1

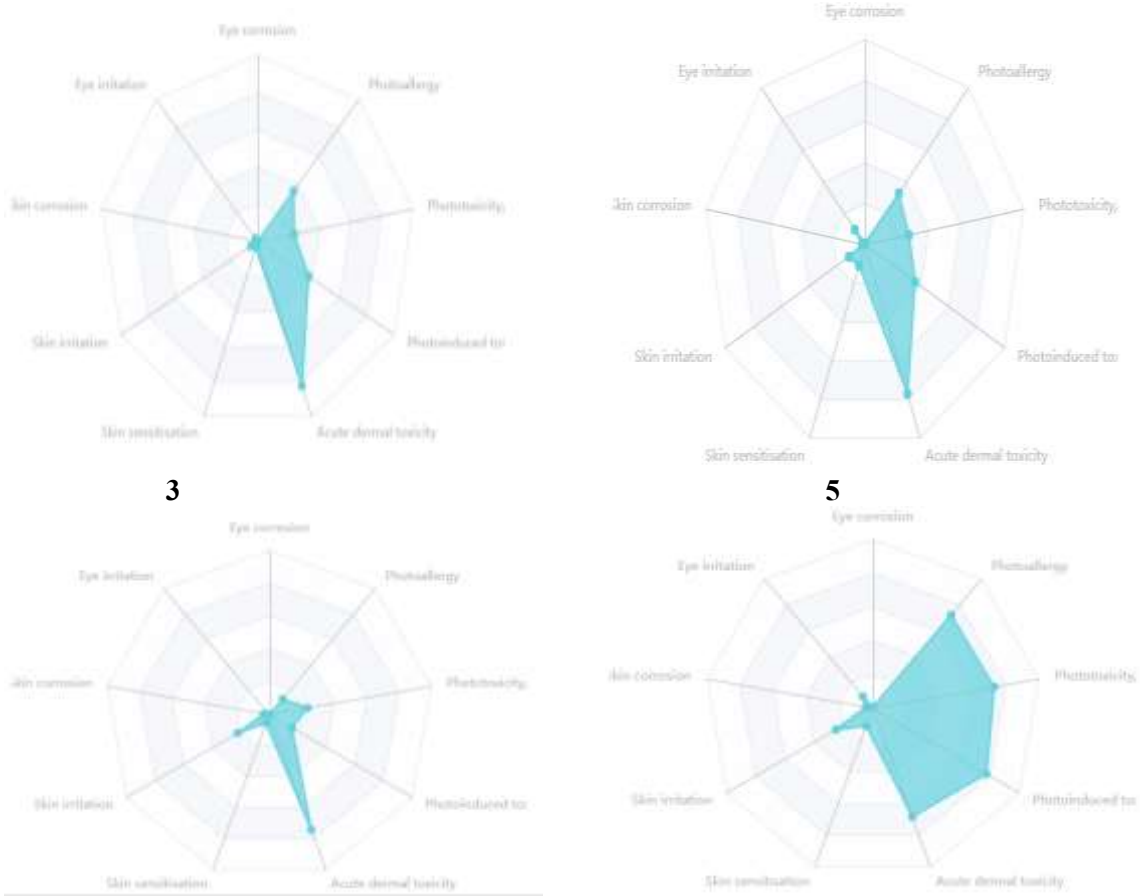


Figure-22: Cosmetic risk assessment view of 3 and 5 along with Amphotericin B and Ciprofloxacin

3.3.11 BOILED-Egg

The BOILED-Egg model is a widely used computational instrument for predicting a compound's ability to penetrate the brain or be taken up in the gastrointestinal system. This straightforward yet effective model categorizes compounds based on molecular features such as lipophilicity (logP) and topological polar surface area (TPSA). The findings are illustrated on a graph, where the yolk area signifies substances likely to pass through the blood-brain barrier, and the white area indicates compounds appropriate for intestinal absorption. The model is particularly appreciated for its simplicity and rapid assessment of a compound's ADME characteristics, which assists researchers in identifying drug candidates with advantageous pharmacokinetics during the initial stages of drug development. Boiled Egg of the synthesized 3 and 5 along with Amphotericin B and Ciprofloxacin were generated by the freely available online web-tool SwissADME.

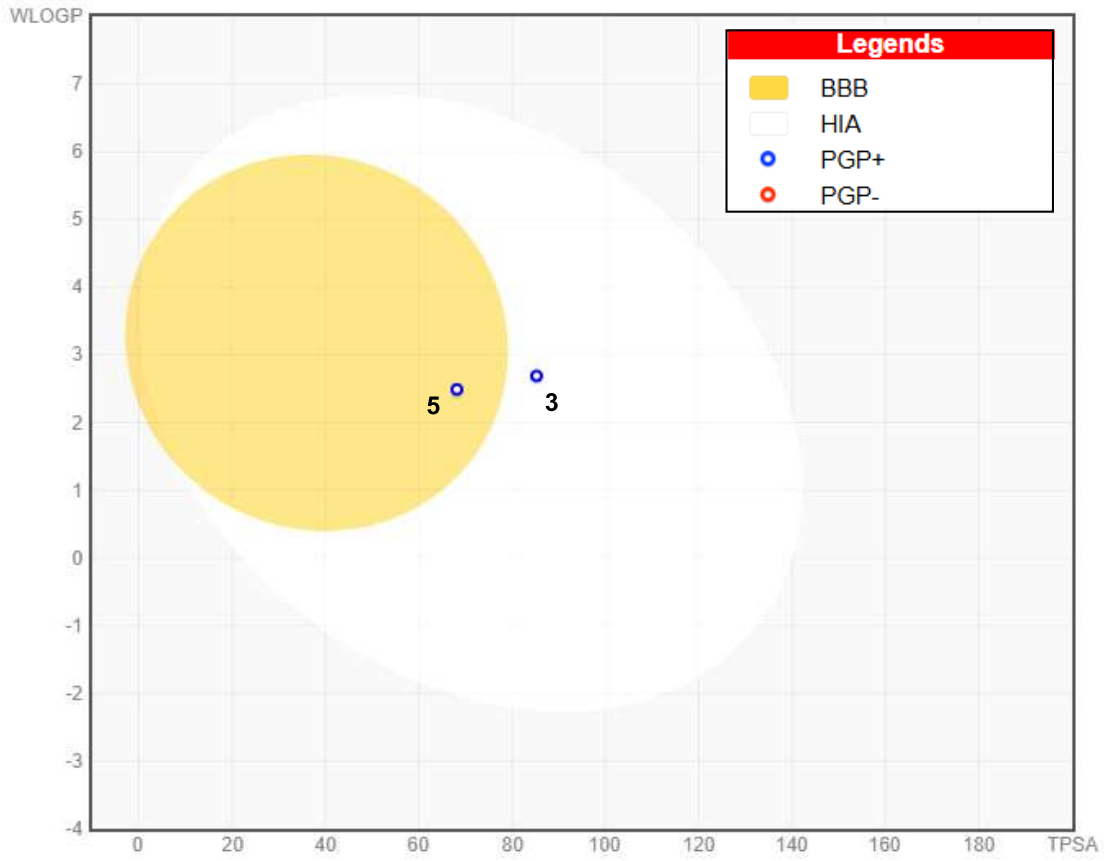


Figure-23: Boiled egg of the synthesized derivative 3 and 5 along with Amphotericin B and Ciprofloxacin

3.4CONCLUSION

Using both in-vitro tests and in-silico predictions, this study successfully synthesized and characterized two new chalcone-based compounds (3 and 5) and assessed their antimicrobial properties. While it showed no significant action against Bacillus subtilis, derivative 5 exhibited superior antibacterial activity, especially against Staphylococcus aureus (14 mm), Escherichia coli (8 mm), and Pseudomonas aeruginosa (9 mm). Derivative 5 showed inhibition zones of 9.33 mm against both Trichoderma asperellum and Candida parapsilosis in antifungal tests; in contrast, derivative 3 showed moderate zones of inhibition up to 8 mm against bacterial strains and up to 9.66 mm against fungal strains. In-silico ADMET and toxicity assessments confirmed favorable pharmacokinetic characteristics and minimal predicted toxicity for both derivatives; compound 5 emerging as the more promising scaffold. These results imply that compound 5 could be a possible lead candidate for the creation of novel broad-spectrum antimicrobial medications, therefore further refinement and in-vivo verification are justified.

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AUTHOR CONTRIBUTIONS:

Mohd Akil: Resources, Writing-original draft preparation
Abdul Rahman Khan: Data Curation, Visualization, Writing-original draft
Firoj Hassan: Conceptualization, Writing-original draft preparation, Visualization
Iqbal Azad: Resources, Supervision, Writing-Review & Editing
Naseem Ahmad: Supervision, Writing-original draft, Supervision, Formal Analysis

CONFLICT OF INTEREST:

The authors declare no competing interests.

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Not applicable.

CONSENT TO PARTICIPATE:
All authors have agreed to participate.

CONSENT TO PUBLISH:
All authors agree upon publication of the present paper.

REFERENCES

1. Qadir, T., Amin, A., Sharma, P. K., Jeelani, I., & Abe, H. (2022). A review on medicinally important heterocyclic compounds. The Open Medicinal Chemistry Journal, 16(1).
2. Kabir, E., & Uzzaman, M. (2022). A review on biological and medicinal impact of heterocyclic compounds. Results in Chemistry, 4, 100606.
3. Yadav, C. S., Krishna, A., Singh, S. P., Kishan, J., Chopra, S., Srivastava, K., ... & Azad, I. (2025). Synthesis, characterization and bio-evaluation of novel series of pyrazoline derivatives as potential antifungal agents. Scientific Reports, 15(1), 1-20.
4. Yadav, C. S., Krishna, A., Verma, V. K., Wani, Z. R., Azad, I., Khan, A. R., & Lohani, M. B. Design, Synthesis and Characterization of 5-(2-Bromo-5-Fluorophenyl)-3-(1h-Pyrrol-2-Yl)-4, 5-Dihydro-1h-Pyrazole as Antifungal Agent. REDVET-Revista electrónica de Veterinaria, 25(1S), 2024.
5. Hassan, F., Azad, I., Asif, M., Shukla, D., Husain, A., Khan, A. R., ... & Nasibullah, M. (2023). Isatin conjugates as antibacterial agents: a brief review. Medicinal Chemistry, 19(5), 413-430.
6. Patnayak, S. (2024). HETEROCYCLIC COMPOUNDS AN POTENTIAL DRUG AND ITS BIOLOGICAL ACTIVTY: A REVIEW. Journal of Nonlinear Analysis and Optimization, 15(1).
7. Baranwal, J., Kushwaha, S., Singh, S., & Jyoti, A. (2023). A review on the synthesis and pharmacological activity of heterocyclic compounds. Current Physical Chemistry, 13(1), 2-19.
8. Kabir, E., & Uzzaman, M. (2022). A review on biological and medicinal impact of heterocyclic compounds. Results in Chemistry, 4, 100606.
9. Kumar, A., & Mishra, A. (2023). ROLE OF HETEROCYCLIC COMPOUNDS IN PHARMACEUTICALS AND MEDICINES. Journal of Experimental Zoology India, 26(1).
10. Ebenezer, O., Jordaan, M. A., Carena, G., Bono, T., Shapi, M., & Tuszynski, J. A. (2022). An overview of the biological evaluation of selected nitrogen-containing heterocycle medicinal chemistry compounds. International Journal of Molecular Sciences, 23(15), 8117.
11. Tran, T. N., & Henary, M. (2022). Synthesis and applications of nitrogen-containing heterocycles as antiviral agents. Molecules, 27(9), 2700.
12. Amin, A., Qadir, T., Sharma, P. K., Jeelani, I., & Abe, H. (2022). A review on the medicinal and industrial applications of N-containing heterocycles. The Open Medicinal Chemistry Journal, 16(1).
13. Mustafa, M., & Winum, J. Y. (2022). The importance of sulfur-containing motifs in drug design and discovery. Expert opinion on drug discovery, 17(5), 501-512.
14. Aksoz, B. E. (2021). Chalcones as Anti-inflammatory, Anti-diabetic, and Anti-depressant Agents. In Frontiers in Natural Product Chemistry: Volume 7 (pp. 197-246). Bentham Science Publishers.
15. Wang, S., Li, C., Zhang, L., Sun, B., Cui, Y., & Sang, F. (2023). Isolation and biological activity of natural chalcones based on antibacterial mechanism classification. Bioorganic & Medicinal Chemistry, 93, 117454.
16. de Oliveira, A. S., Cenci, A. R., Gonçalves, L., Thedy, M. E. C., Justino, A., Braga, A. L., & Meier, L. (2024). Chalcone derivatives as antibacterial agents: An updated overview. Current Medicinal Chemistry, 31(17), 2314-2329.
17. Ali, S. N., Yadav, Khan, M. A., Khan, A. R. & Hassan, F. (2025). A Report On Chalcone Derivatives: Anticancer Effect In Drug Developments. African Journal of Biomedical Research, 28(2S), 245-298. https://doi.org/10.53555/AJBR.v28i2S.6795

18. Constantinescu, T., & Lungu, C. N. (2021). Anticancer activity of natural and synthetic chalcones. International journal of molecular sciences, 22(21), 11306.
19. Kumar, V., Dhawan, S., Girase, P. S., Awolade, P., Shinde, S. R., Karpoormath, R., & Singh, P. (2021). Recent advances in chalcone-based anticancer heterocycles: A structural and molecular target perspective. Current Medicinal Chemistry, 28(33), 6805-6845.
20. Nematollahi, M. H., Mehrabani, M., Hozhabri, Y., Mirtajaddini, M., & Iravani, S. (2023). Antiviral and antimicrobial applications of chalcones and their derivatives: From nature to greener synthesis. Heliyon, 9(10).
21. Ali, S. N., Yadav, C. S., Khan, M. A., Kamal, A., Khan, A. R., Azad, I., ... & Hassan, F. (2025). Synthesis of dioxolylethan-1-one-containing isatin-based chalcone derivatives and their antibacterial activity. American Journal of Psychiatric Rehabilitation, 28(1), 346-359.
22. Dos Santos, A. T. L., de Araújo-Neto, J. B., da Silva, M. M. C., da Silva, M. E. P., Carneiro, J. N. P., Fonseca, V. J. A., ... & Morais-Braga, M. F. B. (2023). Synthesis of chalcones and their antimicrobial and drug potentiating activities. Microbial Pathogenesis, 180, 106129.
23. Chavan, H. V., Ganapure, S. D., Mali, N. N., & Bhale, P. S. (2023). Synthesis, characterization and biological evaluation of N-substituted indolyl chalcones as anticancer, anti-inflammatory and antioxidant agents. Materials Today: Proceedings, 73, 396-402.
24. Mukhtar, S. S., Morsy, N. M., Hassan, A. S., Hafez, T. S., Hassaneen, H. M., & Saleh, F. M. (2022). A review of chalcones: synthesis, reactions, and biological importance. Egyptian Journal of Chemistry, 65(8), 379-395.
25. Sinha, S., Medhi, B., Radotra, B. D., Batovska, D. I., Markova, N., Bhalla, A., & Sehgal, R. (2022). Antimalarial and immunomodulatory potential of chalcone derivatives in experimental model of malaria. BMC complementary medicine and therapies, 22(1), 330.
26. Wulan, F. F., Pranaya, R. C., Nadira, I., Astuti, E., Prasetyo, N., & Wahyuningsih, T. D. (2025). Furanyl-Chalcones as antimalarial agent: synthesis, in vitro study, DFT, and docking analysis of PfDHFR inhibition. Pure and Applied Chemistry, 97(6), 673-688.
27. Taha, M., Sadia, H., Rahim, F., Khan, M. I., Hayat, S., Iqbal, N., ... & Khan, K. M. (2023). Synthesis, biological evaluation and molecular docking study of oxindole based chalcone analogues as potent anti-Alzheimer agents. Journal of Molecular Structure, 1285, 135530.
28. Wang, X. Q., Zhou, L. Y., Tan, R. X., Liang, G. P., Fang, S. X., Li, W., ... & Chen, Y. P. (2021). Design, Synthesis, and Evaluation of Chalcone Derivatives as Multifunctional Agents against Alzheimer's Disease. Chemistry & Biodiversity, 18(11), e2100341.
29. Maschio-Lima, T., Lemes, T. H., Marques, M. D. R., Siqueira, J. P. Z., de Almeida, B. G., Caruso, G. R., ... & de Almeida, M. T. G. (2025). Synergistic activity between conventional antifungals and chalcone-derived compound against dermatophyte fungi and Candida spp. International Microbiology, 28(2), 265-275.
30. Shinde, R. A., Adole, V. A., & Jagdale, B. S. (2022). Synthesis, computational, antibacterial and antifungal investigation of two tri-fluorinated chalcones of 1-(2, 3-Dihydrobenzo [b][1, 4] dioxin-6-yl) ethan-1-one. Polycyclic Aromatic Compounds, 42(9), 6155-6172.
31. Kapoor, K., Kaur, N., Sohal, H. S., Kaur, M., Singh, K., & Kumar, A. (2025). Drugs and Their Mode of Action: A Review on Sulfur-Containing Heterocyclic Compounds. Polycyclic Aromatic Compounds, 45(1), 136-175.
32. He, C., Mao, Y., & Wan, H. (2025). In-depth understanding of the structure-based reactive metabolite formation of organic functional groups. Drug Metabolism Reviews, 57(2), 147-189.
33. John C. Christenson, E. Kent Korgenski, Ryan F. Relich, 286 - Laboratory Diagnosis of Infection Due to Bacteria, Fungi, Parasites, and Rickettsiae, Editor(s): Sarah S. Long, Charles G. Prober, Marc Fischer, Principles and Practice of Pediatric Infectious Diseases (Fifth Edition), Elsevier, 2018, Pages 1422 1434.e3, ISBN 9780323401814
34. Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., ... & Cao, D. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic acids research, 49(W1), W5-W14.
35. Dong, J., Wang, N. N., Yao, Z. J., Zhang, L., Cheng, Y., Ouyang, D., ... & Cao, D. S. (2018). ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. Journal of cheminformatics, 10, 1-11.

36. Duan, Y. J., Fu, L., Zhang, X. C., Long, T. Z., He, Y. H., Liu, Z. Q., ... & Cao, D. S. (2023). Improved GNNs for log D 7.4 prediction by transferring knowledge from low-fidelity data. Journal of Chemical Information and Modeling, 63(8), 2345-2359.
37. Yu, J., Wang, J., Zhao, H., Gao, J., Kang, Y., Cao, D., ... & Hou, T. (2022). Organic compound synthetic accessibility prediction based on the graph attention mechanism. Journal of chemical information and modeling, 62(12), 2973-2986.
38. Dong, J., Wang, N. N., Liu, K. Y., Zhu, M. F., Yun, Y. H., Zeng, W. B., ... & Cao, D. S. (2017). ChemBCPP: a freely available web server for calculating commonly used physicochemical properties. Chemometrics and Intelligent Laboratory Systems, 171, 65-73.
39. Wu, J., Wan, Y., Wu, Z., Zhang, S., Cao, D., Hsieh, C. Y., & Hou, T. (2023). MF-SuP-pKa: Multi-fidelity modeling with subgraph pooling mechanism for pKa prediction. Acta Pharmaceutica Sinica B, 13(6), 2572-2584.
40. Long, T. Z., Shi, S. H., Liu, S., Lu, A. P., Liu, Z. Q., Li, M., ... & Cao, D. S. (2022). Structural analysis and prediction of hematotoxicity using deep learning approaches. Journal of Chemical Information and Modeling, 63(1), 111-125.
41. Yang, Z. Y., Yang, Z. J., Dong, J., Wang, L. L., Zhang, L. X., Ding, J. J., ... & Cao, D. S. (2019). Structural analysis and identification of colloidal aggregators in drug discovery. Journal of chemical information and modeling, 59(9), 3714-3726.
42. Yang, Z. Y., Dong, J., Yang, Z. J., Yin, M., Jiang, H. L., Lu, A. P., ... & Cao, D. S. (2021). ChemFLuo: a web-server for structure analysis and identification of fluorescent compounds. Briefings in bioinformatics, 22(4), bbaa282.
43. Yang, Z. Y., He, J. H., Lu, A. P., Hou, T. J., & Cao, D. S. (2020). Frequent hitters: nuisance artifacts in high-throughput screening. Drug discovery today, 25(4), 657-667.
44. Yang, Z. Y., He, J. H., Lu, A. P., Hou, T. J., & Cao, D. S. (2020). Application of negative design to design a more desirable virtual screening library. Journal of Medicinal Chemistry, 63(9), 4411-4429.
45. Wu, Z., Jiang, D., Wang, J., Hsieh, C. Y., Cao, D., & Hou, T. (2021). Mining toxicity information from large amounts of toxicity data. Journal of Medicinal Chemistry, 64(10), 6924-6936.
46. Jiang, D., Lei, T., Wang, Z., Shen, C., Cao, D., & Hou, T. (2020). ADMET evaluation in drug discovery. 20. Prediction of breast cancer resistance protein inhibition through machine learning. Journal of Cheminformatics, 12, 1-26.
47. Yang, Z. Y., Yang, Z. J., Lu, A. P., Hou, T. J., & Cao, D. S. (2021). Scopy: an integrated negative design python library for desirable HTS/VS database design. Briefings in Bioinformatics, 22(3), bbaa194.
48. Fu, L., Liu, L., Yang, Z. J., Li, P., Ding, J. J., Yun, Y. H., ... & Cao, D. S. (2019). Systematic modeling of log d 7.4 based on ensemble machine learning, group contribution, and matched molecular pair analysis. Journal of Chemical Information and Modeling, 60(1), 63-76.
49. Wu, Z., Lei, T., Shen, C., Wang, Z., Cao, D., & Hou, T. (2019). ADMET evaluation in drug discovery. 19. Reliable prediction of human cytochrome P450 inhibition using artificial intelligence approaches. Journal of chemical information and modeling, 59(11), 4587-4601.
50. Liu, L., Fu, L., Zhang, J. W., Wei, H., Ye, W. L., Deng, Z. K., ... & Cao, D. S. (2018). Three-level hepatotoxicity prediction system based on adverse hepatic effects. Molecular pharmaceutics, 16(1), 393-408.
51. Lei, T., Sun, H., Kang, Y., Zhu, F., Liu, H., Zhou, W., ... & Hou, T. (2017). ADMET evaluation in drug discovery. 18. Reliable prediction of chemical-induced urinary tract toxicity by boosting machine learning approaches. Molecular pharmaceutics, 14(11), 3935-3953.
52. Wang, N. N., Deng, Z. K., Huang, C., Dong, J., Zhu, M. F., Yao, Z. J., ... & Cao, D. S. (2017). ADME properties evaluation in drug discovery: Prediction of plasma protein binding using NSGA-II combining PLS and consensus modeling. Chemometrics and Intelligent Laboratory Systems, 170, 84-95.
53. Wang, N. N., Huang, C., Dong, J., Yao, Z. J., Zhu, M. F., Deng, Z. K., ... & Cao, D. S. (2017). Predicting human intestinal absorption with modified random forest approach: a comprehensive evaluation of molecular representation, unbalanced data, and applicability domain issues. RSC advances, 7(31), 19007-19018.

54. Lei, T., Chen, F., Liu, H., Sun, H., Kang, Y., Li, D., ... & Hou, T. (2017). ADMET evaluation in drug discovery. Part 17: development of quantitative and qualitative prediction models for chemical-induced respiratory toxicity. Molecular pharmaceutics, 14(7), 2407-2421.
55. Wang, S., Sun, H., Liu, H., Li, D., Li, Y., & Hou, T. (2016). ADMET evaluation in drug discovery. 16. Predicting hERG blockers by combining multiple pharmacophores and machine learning approaches. Molecular pharmaceutics, 13(8), 2855-2866.
56. Wang, N. N., Dong, J., Deng, Y. H., Zhu, M. F., Wen, M., Yao, Z. J., ... & Cao, D. S. (2016). ADME properties evaluation in drug discovery: prediction of Caco-2 cell permeability using a combination of NSGA-II and boosting. Journal of chemical information and modeling, 56(4), 763-773.
57. Lei, T., Li, Y., Song, Y., Li, D., Sun, H., & Hou, T. (2016). ADMET evaluation in drug discovery: 15. Accurate prediction of rat oral acute toxicity using relevance vector machine and consensus modeling. Journal of cheminformatics, 8, 1-19.