

DESIGN, SYNTHESIS, MOLECULAR DOCKING, ADME PREDICTION, AND BIOLOGICAL EVALUATION OF PYRIMIDINE-2-THIOL DERIVATIVES

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ABSTRACT

The growing problem of antimicrobial resistance has created a need for new agents; a new class of pyrimidine derivatives was synthesized, and their biological potential was determined by *in vitro* and *in silico* analyses. Pyrimidine derivatives were designed and synthesized in the wet lab. The Structures of the compounds were confirmed by IR, ¹H, ¹³C NMR, and CHN analysis. The synthesized compounds were further evaluated through molecular docking against alpha-glycerophosphate oxidase, *in silico* ADME prediction, antimicrobial assay by agar well diffusion and minimum inhibitory concentration (MIC) assays, antioxidant activity using the DPPH method, and *in vitro* anticancer evaluation against the MDA-MB-231 human breast cancer cell line using the sulforhodamine B (SRB) assay. The ADME analysis indicated that most compounds possessed favourable drug-like properties, with good predicted oral absorption and compliance with Lipinski's rule of five. Antimicrobial studies demonstrated concentration-dependent activity. Among the tested compounds, M5 displayed the strongest antibacterial activity, while C9, M5, C11, and M3 showed the lowest MIC values (7.8125–31.25 µg/ml). In the antioxidant assay, derivatives containing electron-donating substituents exhibited better free-radical scavenging activity than the remaining compounds. In addition, M4 showed the highest antiproliferative activity against MDA-MB-231 cells with a GI₅₀ value of 5 µM, followed by M1 (GI₅₀ = 8 µM). These findings identify this scaffold as a promising lead for the further development of a multifunctional therapeutic agent.

KEYWORDS: ADME, Antimicrobial, Antioxidant, Chalcone, alpha-glycerophosphate oxidase, Pyrimidine, Molecular docking

INTRODUCTION

The continuous search for biologically active molecules has made heterocyclic chemistry one of the most dynamic areas of medicinal chemistry. The presence of nitrogen, oxygen, and sulfur atoms within heterocyclic frameworks confers unique structural and electronic properties that facilitate interactions with a diverse range of biological targets. As a result, heterocyclic scaffolds are widely represented in approved pharmaceuticals and continue to serve as valuable templates for the discovery of new agents against infectious diseases, cancer, inflammatory disorders, and other human diseases 1–3. Pyrimidine is one of the most extensively investigated heterocyclic scaffolds due to its structural diversity and wide spectrum of pharmacological activities. The broad pharmacological profile of pyrimidines arises from their ability to interact with diverse enzyme systems through hydrogen bonding and π -interactions. The therapeutic importance of the pyrimidine nucleus is evident from its presence in several clinically approved drugs, including fluorouracil, gemcitabine, etravirine, rosuvastatin, and iclaprim. These findings highlight the significance of pyrimidine derivatives as promising scaffolds for the design and development of new therapeutic agents 4–10.

Although antimicrobial agents have significantly improved the treatment of bacterial infections, the increasing prevalence of antimicrobial resistance (AMR) has become a major global health concern. Excessive and inappropriate use of antibiotics has contributed to the emergence and spread of resistant bacterial strains, thereby reducing the clinical efficacy of many existing antimicrobial drugs. Multidrug-resistant pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*, are now responsible for a growing number of difficult-to-treat infections. This situation underscores the need to develop structurally diverse antibacterial agents with enhanced potency and improved safety profiles 11–14.

Chalcones are well-known bioactive molecules possessing a broad range of pharmacological activities, including antimicrobial, antioxidant, antidiabetic, and anticancer effects. Their biological activity can be further improved by introducing suitable electron-donating or electron-withdrawing substituents and by combining them with biologically active heterocyclic scaffolds. In particular, molecular hybridization of chalcone with the pyrimidine nucleus has attracted considerable attention as an effective strategy for developing multifunctional therapeutic agents. This approach formed the basis for designing the pyrimidine–chalcone derivatives investigated in the present study 15–17.

Alpha-glycerophosphate oxidase (GlpO) is a flavin adenine dinucleotide (FAD)-dependent oxidoreductase involved in glycerol-3-phosphate metabolism. The enzyme catalyses the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate, with hydrogen peroxide generated as a reaction product. In certain bacterial species, hydrogen peroxide production may contribute to host-cell damage and bacterial virulence. The crystal structure of GlpO fr. is available in the

Protein Data Bank under accession code 2RGO, with a reported resolution of 2.40 Å. This experimentally determined structure provides a structural framework for investigating potential ligand-binding interactions through molecular docking studies. Molecular docking studies against alpha-glycerophosphate oxidase (GlpO; PDB ID: 2RGO) can investigate predicted binding interactions of pyrimidine derivatives and support the computational assessment of potential antibacterial candidates before experimental evaluation 18–20.

Although several pyrimidines and chalcone derivatives have been reported with antimicrobial and other pharmacological activities, comprehensive studies integrating rational molecular design, molecular docking, ADME prediction, and multiple biological evaluations within a single series of pyrimidine-2-thiol derivatives remain limited. Moreover, researchers have not systematically investigated how different aromatic substitutions affect their biological activity. Therefore, the present study was designed to develop new pyrimidine-2-thiol derivatives and evaluate their antimicrobial, antioxidant, and anticancer potential through an integrated computational and experimental approach.

In the present study, a series of substituted pyrimidine-2-thiol derivatives was synthesized using appropriately substituted chalcones as key intermediates. The structures of the synthesized compounds were confirmed by FT-IR, ¹H NMR, and ¹³C NMR spectroscopy. Their antibacterial activity was assessed against selected Gram-positive and Gram-negative bacterial strains, whereas antioxidant activity was evaluated by the DPPH free-radical scavenging assay. In addition, molecular docking studies and in silico ADME analysis were performed to examine the predicted binding behaviour and pharmacokinetic characteristics of the synthesized compounds. Finally, structure–activity relationship (SAR) analysis was carried out to identify the structural features associated with the observed biological activities and to recognize promising lead molecules for further optimization. A series of new pyrimidine-2-thiol derivatives was synthesized using substituted chalcones as key intermediates.

2. Rationale and Design Strategy

The design of the target pyrimidine derivatives followed a rational, structure-based strategy. Initially, a comprehensive literature survey was conducted to identify previously reported pyrimidine derivatives exhibiting significant antimicrobial activity. The reported active compounds were then subjected to molecular docking studies against alpha-glycerophosphate oxidase (GlpO, PDB ID: 2RGO) along with in silico ADME evaluation to identify the most promising lead compound. Based on the docking interactions and predicted pharmacokinetic properties, the selected lead structure was further optimized by introducing different electron-donating and electron-withdrawing substituents on the aromatic ring while retaining the 4-methoxyacetophenone-derived chalcone framework 16. The newly designed derivatives were again evaluated by molecular docking and ADME analysis. Based on these results, the final set of compounds was selected for synthesis and subsequent antibacterial and antioxidant evaluation (Fig. 1).

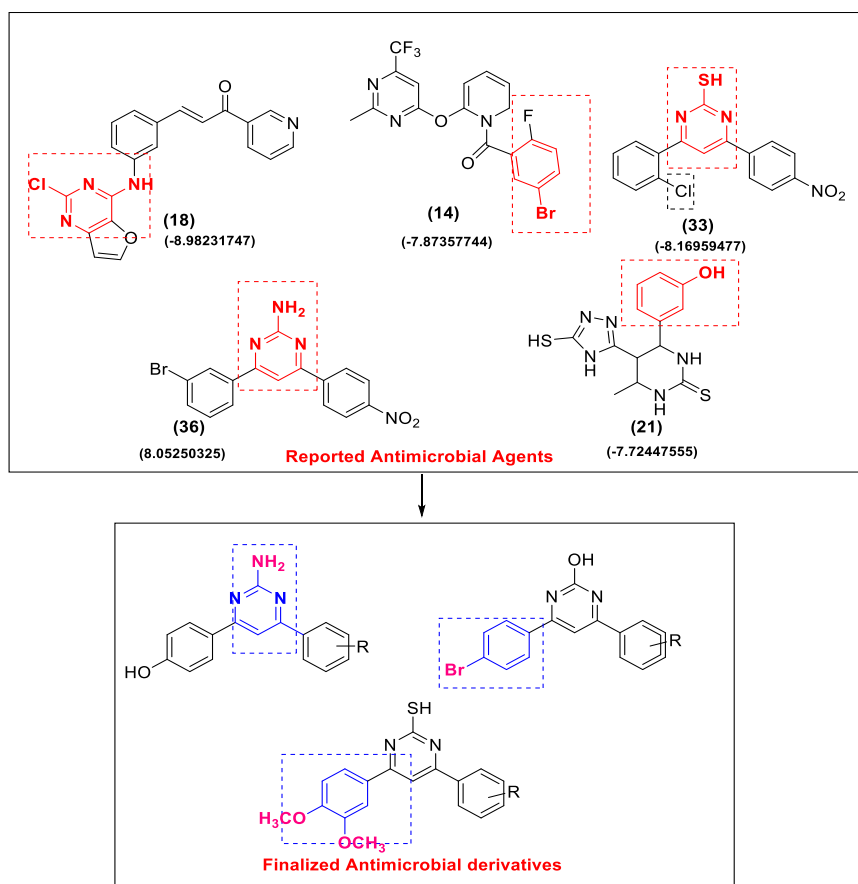


Fig. 1: Design of the proposed pyrimidine derivatives was based on a literature review and analysis of molecular docking scores.

3. Experimental section:

3.1. Materials and methods:

Materials, reagents, and solvents used for synthesizing pyrimidine derivatives were obtained from CDH Chemicals, New Delhi, India and Loba Chemie Pvt. Ltd., Mumbai, India and used without further purification. The progress of the reaction was monitored by thin-layer chromatography (TLC) using Merck pre-coated TLC plates (Silica gel 60 F254). The melting points of the synthesized compounds were determined in the open capillary tube method on a digital melting point apparatus. Infrared spectra were recorded on a Bruker FT-IR spectrometer using OPUS software. ¹H and ¹³C NMR spectra were recorded by Bruker 500 MHz NMR spectrometer in DMSO-d₆ solvents and given as multiple peaks (s, singlet; d, doublet; t, triplet; m, multiplet) of a number of protons. An ultraviolet visible spectrophotometer of Labindia UV 3000 was used to obtain absorbance for antioxidant activity.

3.2. General procedure for the synthesis of pyrimidine derivatives:

3.2.1. Synthesis of intermediate I (Chalcone): A methanolic solution of (0.01 mol) of 4-methoxyacetophenone and (0.01mol) appropriate benzaldehyde was prepared in a 100 ml conical flask. After that, 10% NaOH was added dropwise to it, and the resulting mixture was stirred for 30-40 minutes at 20-25 °C. The progress of the reaction was observed using TLC in benzene and methanol in a ratio of 8:2 (v/v) as the mobile phase. The reaction mixture was allowed to stand overnight, and the resulting precipitate was collected by filtration, washed with cold water, and then dried. Then precipitate was recrystallized using ethanol to maximize the yield of the final product (C1-C14) ²¹.

3.2.2. Synthesis of derivatives (M1-M14):

To synthesize pyrimidine derivatives, a methanolic solution of chalcone (0.01 mol) and thiourea (0.01 mol) was dissolved in a round-bottom flask. The mixture was further refluxed in the presence of acetic acid for 6–8 h with continuous stirring. The progress of the reaction was observed by TLC using hexane and ethyl acetate in a ratio of 2:5 (v/v) as the mobile phase till the appearance of a single spot corresponding to the desired product. After completion of the reaction, the reaction mixture was cooled in ice-cold water, the precipitate was filtered, and recrystallization was performed using absolute methanol ²². The synthesized compounds predominantly exist in the thiol form due to thione–thiol tautomerism, which is consistent with the observed spectroscopic data.

The structure of these compounds was characterized using physicochemical and spectral IR, ¹H NMR, and ¹³C NMR analysis.

3.3. Spectral characterization

(E)-3-(4-bromophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C1): 3072(C–H str.), 2962(C–H str., –OCH₃), 1654(C=O str.), 1596(C=C str.), 1254(C–O str., Ar–OCH₃), 1172(C–O–C str.), 810(C–H bend.), 622(C–Br str.), ¹H-NMR (CDCl₃, δ, ppm): 6.85–7.35 (d, 8H, Ar–H), 7.57, 7.85 (s, 2H, CH of pyrimidine ring), 3.83 (s, 3H, OCH₃), ¹³C NMR: aromatic nucleus: (127.2, 129.4, 134.5, 136.7), 161.2(C–O), OCH₃-(55.7), 192.1(C=O, –Chalcone).

(E)-3-(3-bromophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C2): 2979(C–H str., –OCH₃), 1670(C=O str.), 1588(C=C str.), 1263(C–O str., Ar–OCH₃), 1057(C–O–C str.), 882(C–H bend.), 687(C–Br str.); ¹H NMR (DMSO-d₆, δ ppm): 3.85 (s, 3H, OCH₃), 7.27–7.66 (m, 8H, Ar–H), 7.84 (d, 1H, CH=CH), 8.03 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.8 (OCH₃), 113.6, 122.6, 125.8, 128.6, 130.2, 131.7, 133.8, 136.4 (aromatic carbons), 160.9 (Ar–O), 190.9 (C=O).

(E)-3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C3): 3182(C–H str.), 2965(C–H str., –OCH₃), 1669(C=O str.), 1597(C=C str.), 1255(C–O str., Ar–OCH₃), 1175(C–O–C str.), 809(C–H bend.), 676(C–Cl str.) ¹H NMR (CDCl₃, δ ppm): 3.83 (s, 3H, OCH₃), 6.88–7.62 (m, 8H, Ar–H), 7.84 (d, 1H, CH=CH), 8.03 (d, 1H, CH=CH,); ¹³C NMR (δ ppm): 55.8 (OCH₃), 114.2, 124.8, 127.9, 129.3, 130.6, 131.8, 134.4, 160.6 (aromatic carbons), 190.7 (C=O).

(E)-3-(3-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C4): 3182(C–H str.), 2359(C–H str., –OCH₃), 1634(C=O str.), 1679(C=C str.), 1250(C–O str., Ar–OCH₃), 1192(C–O–C str.), 748(C–H bend.), 679(C–Cl str.); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 6.86–7.68 (m, 8H, Ar–H), 7.83 (d, 1H, CH=CH), 8.02 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.8 (OCH₃), 114.3, 123.9, 126.8, 128.7, 129.9, 130.8, 133.6, 135.4, 160.4 (aromatic carbons), 190.8 (C=O).

(E)-3-(2-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C5): 3064(C–H str.), 2359(C–H str., –OCH₃), 1595(C=O str.), 1664(C=C str.), 1253(C–O str., Ar–OCH₃), 1166(C–O–C str.), 974(C–H str.), 752(C–H bend.), 681(C–Cl str.); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 6.89–7.72 (m, 8H, Ar–H), 7.82 (d, 1H, CH=CH), 8.02 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.8 (OCH₃), 113.9, 120.8, 126.9, 127.4, 129.0, 129.8, 130.6, 132.8, 135.1, 161.5 (aromatic/olefinic carbons), 190.7 (C=O).

(E)-1,3-bis(4-methoxyphenyl)prop-2-en-1-one (6): 2972(C–H str.), 2837(C–H str., –OCH₃), 1664(C=O str.), 1600(C=C str.), 1511(C–C str.), 1258(C–O str., Ar–OCH₃), 1115(C–O–C str.), 1027(=C–H bend.), 832(Para-substituted benzene ring); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 6H, 2 × OCH₃), 6.90 (d, J ≈ 8.7 Hz, 4H, Ar–H), 7.48 (d, 1H, CH=CH), 7.61 (d, 1H, CH=CH), 7.96 (d, J ≈ 8.7 Hz, 4H, Ar–H); ¹³C NMR (δ ppm): 55.7 (2 × OCH₃), 113.9, 120.8, 127.8, 129.5, 130.1, 144.2, 161.8 (aromatic/olefinic carbons), 190.2 (C=O).

(E)-1-(4-methoxyphenyl)-3-(p-tolyl)prop-2-en-1-one (7): 2963(C–H str., –OCH₃), 2837(C–H str., –OCH₃), 1674(C=C str.), 1600(C=O str.), 1511(C–C str.), 1258(C–O str., Ar–OCH₃), 1173(C–O–C str.), 1027(=C–H bend.), 836(Para-substituted benzene ring); ¹H NMR (CDCl₃, δ ppm): 2.37 (s, 3H, Ar–CH₃), 3.84 (s, 3H, OCH₃), 6.90 (d, J ≈ 8.7 Hz, 2H, Ar–H), 7.24 (d, J ≈ 8.1 Hz, 2H, Ar–H), 7.48 (d, 1H, CH=CH), 7.62 (d, 1H, CH=CH), 7.96 (d, J ≈ 8.7 Hz, 2H, Ar–H); ¹³C NMR (δ ppm): 21.5 (Ar–CH₃), 55.7 (OCH₃), 113.9, 120.7, 127.6, 129.4, 129.8, 130.2, 143.8, 161.7 (aromatic), 190.5 (C=O).

(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (8): 3440 (O–H str.), 2928 (C–H str.), 2837 (C–H str., –OCH₃), 1658 (C=O str.), 1602 (C=C str.), 1510 (C–C str., aromatic ring), 1260 (C–O str., Ar–OCH₃), 1120 (C–O–C str.), 1028 (=C–H bend.), 835 (C–H bend., para-substituted benzene ring); ¹H-NMR (CDCl₃, δ, ppm): 3.80 (s, 6H, OCH₃), 3.90 (s, 3H, OCH₃), 6.20 (s, Ar–1H), 6.82–7.74 (m, Ar–H), 7.88 (d, 1H), 8.09 (d, 1H), ¹³C NMR: aromatic nucleus: 55, 60, 100–160 (Ar–C, C=C), ~190 (C=O); ¹³C NMR (δ ppm): 55.7 (OCH₃), 56.2 (2 × OCH₃), 108.5, 113.9, 120.5, 127.8, 129.6, 139.8, 147.2, 153.1, 161.7 (aromatic), 190.4 (C=O).

(E)-3-(3-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (9): FTIR (cm⁻¹): 3392 (O–H stretching), 3062 (Ar–C–H stretching), 2958 (C–H stretching, –OCH₃), 1658 (C=O stretching), 1598 (C=C stretching), 1256 (Ar–OCH₃ stretching), 1174 (C–O–C stretching), 1138 (C–O stretching, phenolic OH), 826 (Ar–H bending); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 6.82–7.68 (m, 8H, Ar–H), 7.82 (d, 1H, CH=CH), 8.05 (d, 1H, CH=CH), 9.72 (s, 1H, OH); ¹³C NMR (δ ppm): 55.8 (OCH₃), 113.9, 115.8, 121.7, 124.6, 127.8, 129.5, 130.8, 145.6, 159.8 (aromatic carbons), 190.5 (C=O).

(E)-1-(4-methoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (10): 2926(C–H str., –OCH₃), 2854(C–H str., –OCH₃), 1602(C=C str.), 1650(C–O str.), 1517(C–C str.), 1265(C–O str., Ar–OCH₃), 1055(C–O–C str.), 1021(=C–H bend.), 1540(str., –NO₂); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 7.00–8.15 (m, 8H, Ar–H), 7.72 (d, 1H, CH=CH), 8.02 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.7 (OCH₃), 113.9, 124.8, 126.7, 128.9, 129.5, 130.4, 132.8, 135.6, 148.1, 161.8 (aromatic/olefinic carbons), 189.8 (C=O).

(E)-1-(4-methoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (11): 2971(C–H str., –OCH₃), 2866(C–H str., –OCH₃), 1616(C=C str.), 1649(C–O str.), 1519(C–C str.), 1261(C–O str., Ar–OCH₃), 1055(C–O–C str.), 1015(=C–H bend.), 1519(str., –NO₂); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 6.92 (d, J ≈ 8.7 Hz, 2H, Ar–H), 7.48 (d, 1H, CH=CH), 7.64 (d, 1H, CH=CH), 7.95 (d, J ≈ 8.7 Hz, 2H, Ar–H), 8.15 (d, J ≈ 8.7 Hz, 2H, Ar–H), 8.28 (d, J ≈ 8.7 Hz, 2H, Ar–H); ¹³C NMR (δ ppm): 55.7 (OCH₃), 113.9, 124.2, 128.1, 129.6, 130.3, 141.7, 148.0, 161.8 (aromatic/olefinic carbons), 189.7 (C=O).

(E)-1-(4-methoxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (12): 2971(C–H str., –OCH₃), 2867(C–H str., –OCH₃), 1606(C=C str.), 1664(C–O str.), 1518(C–C str.), 1260(C–O str., Ar–OCH₃), 1055(C–O–C str.), 1016(=C–H bend.), 1518(str., –NO₂); ¹H NMR (CDCl₃, δ ppm): 3.84 (s, 3H, OCH₃), 6.92 (d, J ≈ 8.7 Hz, 2H, Ar–H), 7.45–8.25 (m, 6H, Ar–H), 7.52 (d, 1H, CH=CH), 7.67 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.7 (OCH₃), 113.9, 121.7, 124.2, 129.0, 129.7, 130.5, 134.2, 140.1, 148.2, 161.8 (aromatic/olefinic carbons), 189.8 (C=O).

(E)-3-(3-aminophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (13): FTIR (cm⁻¹): 3448, 3352 (N–H stretching, –NH₂), 3058 (Ar–C–H stretching), 2956 (C–H stretching, –OCH₃), 1659 (C=O stretching), 1596 (C=C stretching), 1260 (Ar–OCH₃ stretching), 1172 (C–O–C stretching), 1615 (N–H bending), 824 (Ar–H bending); ¹H NMR (DMSO-d₆, δ ppm): 3.82 (s, 3H, OCH₃), 5.18 (s, 2H, NH₂), 6.62–7.58 (m, 8H, Ar–H), 7.84 (d, 1H, CH=CH), 8.02 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 55.7 (OCH₃), 114.4, 116.3, 120.8, 123.7, 127.5, 129.8, 131.5, 147.8, 160.2 (aromatic carbons), 190.8 (C=O).

(E)-3-(3-(dimethylamino)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C14): FTIR (cm⁻¹): 3055 (Ar–C–H stretching), 2945 (C–H stretching), 1662 (C=O stretching), 1600 (C=C stretching), 1510 (Ar C=C), 1260 (Ar–OCH₃ stretching), 1175 (C–O–C stretching), 1218 (C–N stretching), 2812 (N(CH₃)₂ stretching), 824 (Ar–H bending); ¹H NMR (CDCl₃, δ ppm): 2.95 (s, 6H, N(CH₃)₂), 3.83 (s, 3H, OCH₃), 6.70–7.65 (m, 8H, Ar–H), 7.84 (d, 1H, CH=CH), 8.02 (d, 1H, CH=CH); ¹³C NMR (δ ppm): 40.6 (N(CH₃)₂), 55.8 (OCH₃), 112.8, 115.2, 122.4, 126.8, 128.9, 130.4, 151.5, 160.3 (aromatic carbons), 190.7 (C=O).

4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M1): M. weight: 373.27; M. Point (°C): 134–136; Rf: 0.58; % yield: 70; FTIR cm⁻¹: 2875 (C–H), 2637 (S–H), 1671 (C=N), 1597 (Ar C=C), 1355 (C–N), 1175 (Ar–O–CH₃), 834 (para-disubstituted Ar–H), 668 (C–Br); ¹H-NMR DMSO-d₆, δ ppm: 3.8 (s, 3H, OCH₃), 7.0–8.3 (m, 8H, Ar–H), 8.40 (s, 1H, pyrimidine-CH), 3–5 or 11–13 (s, 1H, SH); ¹³C NMR: aromatic nucleus: (55.7 (OCH₃), 114.9, 123.4, 124.6, 124.8, 127.9, 129.6, 131.3, 132.8, 136.4, 160.8, 163.5, 166.7 (Ar and pyrimidine carbons). CHNS (%): Found: C, 54.71; H, 3.55; N, 7.46; S, 8.12.

4-(3-bromophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M2): M. weight: 373.27; M. Point (°C): 140–142; Rf: 0.72; % yield: 86; FTIR (cm⁻¹): 3145 (C–H stretching), 2635 (S–H stretching), 1668 (C=N stretching of pyrimidine), 1601 (aromatic C=C stretching), 1356 (C–N stretching), 1177 (Ar–O–CH₃ stretching), 729 (aromatic C–H out-of-plane bending), 623 (C–Br stretching); ¹H-NMR DMSO-d₆, δ ppm: 3145 (C–H stretching), 2635 (S–H stretching), 1668 (C=N stretching of pyrimidine), 1601 (aromatic C=C stretching), 1356 (C–N stretching), 1177 (Ar–O–CH₃ stretching), 729 (aromatic C–H out-of-plane bending), 623 (C–Br stretching); ¹³C NMR: 55.9 (OCH₃), 114.5, 123.3, 124.2, 127.8, 129.4, 131.1, 132.3, 136.2, 160.7, 163.4, 166.5 (aromatic and pyrimidine carbons); CHNS Analysis (%): Found: C, 54.71; H, 3.55; N, 7.46; S, 8.12.

4-(4-chlorophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M3): M. weight: 328.81; M. Point (°C): 102–104; Rf: 0.71; % yield: 85; IR (cm⁻¹) 2969 (C–H stretch), 1599 (C=C stretch), 725 (C–C stretch), 1352 (C–N stretch, pyrimidine), 816 (C–Cl stretch), 1175 (C–O–C stretch, –OCH₃), 680 (C–N stretch), 680 (C–S stretch), ¹H-NMR DMSO-d₆, δ ppm: 7.08–8.46 (m, 9H, Ar–H), 7.41 (s, 1H, CH of pyrimidine ring), 3.41 (s, 1H, SH), 3.82, 3.86 (s, 3H, –OCH₃), ¹³C NMR: aromatic nucleus: (129.3, 123.2, 114.5, 130.7), pyrimidine nucleus: 163.7, (C4 & C6 attached ring), (131.4, 134.2, 135.3), OCH₃- (56.0), 142.1 (connected to pyrimidine),

4-(3-chlorophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M4): M. weight: 328.81; M. Point (°C): 118–120; Rf: 0.82; % yield: 88; IR (cm⁻¹): 2969 (C–H stretch), 1599 (C=C stretch), 725 (C–C stretch), 1352 (C–N stretch, pyrimidine), 816 (C–Cl stretch), 1175 C–O–C stretch, –OCH₃), 680 (C–N stretch), 680 (C–S stretch), ¹H-NMR DMSO-d₆, δ ppm: 7.03–8.20 (m, 9H, Ar–H), 7.42 (s, 1H, CH of pyrimidine ring), 3.40 (s, 1H, SH), 3.68, 3.86 (s, 3H, –OCH₃), ¹³C NMR: aromatic nucleus: (125.3, 128.9, 130.9, 132.8, 138.2), pyrimidine nucleus: (163.9, 114.2), OCH₃- (55.9)

4-(2-chlorophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M5): M. weight: 328.81; M. Point (°C): 96–98; Rf: 0.79; % yield: 66; IR (cm⁻¹): 2956 (C–H stretch), 1606 (C=C stretch), 723 (C–C stretch), 1679 (C=N stretch or N=CH stretch, pyrimidine ring), 1363 (C–N stretch, pyrimidine), 723 (C–Cl stretch), 841 (C–N stretch), 3384 (NH₂, asym stretch), ¹H-NMR DMSO-d₆, δ ppm: 7.07–8.20 (m, 9H, Ar–H), 7.45 (s, 1H, CH of pyrimidine ring), 3.41 (s, 1H, SH), 3.83, 3.87 (s, 3H, –OCH₃), ¹³C NMR: aromatic nucleus: (124.0, 126.3, 128.4, 130.9, 133.2, 137.5), pyrimidine nucleus: (163.5, 114.2), OCH₃- (55.8)

4,6-bis(4-methoxyphenyl)pyrimidine-2-thiol (M6): M. weight: 324.40; M. Point (°C): 82–84; Rf: 0.62; % yield: 71; IR (cm⁻¹): 2963 (C–H stretch), 1674 1606 (C=C stretch), 1600, 1258 (C–N stretch, pyrimidine), 1173, 1112, 836 (C=C

bend.), 749, 668 (C–S stretch), ¹H-NMR DMSO-*d*₆, δ ppm: 7.66–8.27 (m, 8H, Ar–H), 7.26 (s, 1H, CH of pyrimidine ring), 3.47 (s, 1H, SH), 3.80 (s, 6H, –OCH₃), ¹³C NMR: (127.9, 129.2, 130.7, 130.9, 131.2, 132.5), pyrimidine nucleus: (163.6, 114.3), OCH₃–(55.8, 56.0)

4-(4-methoxyphenyl)-6-(*p*-tolyl)pyrimidine-2-thiol (M7): M. weight: 308.40; M. Point (°C): 102–104; Rf: 0.64; % yield: 46; IR (cm^{–1}): 2972 (C–H stretch), 1651 (C=N stretch or N=CH stretch, pyrimidine ring), 1593, 1502, 1411 (C–H bend. Methyl group), 1302 C–N stretch, pyrimidine), 1210 (C–S–C), 1022, 805 (C=C bend.), 670 (C–S stretch) ¹H-NMR DMSO-*d*₆, δ ppm: 7.74–8.27 (m, 8H, Ar–H), 7.28 (s, 1H, CH of pyrimidine ring), 3.40 (s, 1H, SH), 3.82, 3.83 (s, 6H, –OCH₃). ¹³C NMR: (127.9, 129.2, 130.0, 130.9, 131.3, 132.5), pyrimidine nucleus: (114.3, 114.4, 119.9, 121.4), OCH₃–(55.8, 56.0),

4-(2-mercapto-6-(4-methoxyphenyl)pyrimidin-4-yl)-2,6-dimethoxyphenol (M8): M. weight: 370.42; M. Point (°C): 62–64; Rf: 0.74; % yield: 56; IR (cm^{–1}): 3681 (–OH, phenol), 1259 (C–O–C stretch, –OCH₃) 2828 (C–H stretch), 2558 (C–S stretch), 1694 (C=N stretch or N=CH stretch, pyrimidine ring), 1464 (C–N stretch, pyrimidine), ¹H-NMR DMSO-*d*₆, δ ppm: 7.74–8.27 (m, 7H, Ar–H), 7.28 (s, 1H, CH of pyrimidine ring), 3.40 (s, 1H, SH), 3.82, 3.83 (s, 9H, –OCH₃), 6.93–7.43 (m, 1OH), ¹³C NMR: (123.4, 124.3, 128.7, 130.5, 131.6, 140.7, 146.6, 147.5, 148.4), pyrimidine nucleus: (114.3, 114.6, 163.5), OCH₃–(55.9, 56.1)

3-(2-mercapto-6-(4-methoxyphenyl)pyrimidin-4-yl)phenol (M9): M. weight: 310.37; M. Point (°C): 78–80; Rf: 0.82; % yield: 68; IR (cm^{–1}): 3345 (N–H stretch, –NH₂), 1621 (N–H bend., –NH₂), 2860 (O–C–H stretch, –OCH₃), 1263 (C–O stretch, –OCH₃), 1596 (C=N, –Pyrimidine ring), 2975 (C–H stretch, –pyrimidine ring), 1365 (C–H bend. –pyrimidine), 3348 (O–H stretch), 1146 (C–O stretch, –OH), 3036 (C–H stretch, –Ar), 1528 (C=C stretch); ¹H-NMR DMSO-*d*₆, δ ppm: 7.13–8.34 (m, 8H, Ar–H), 8.94 (s, 1H, CH of pyrimidine ring), 3.46 (s, 1H, SH), 3.81 (s, 3H, –OCH₃); ¹³C NMR: 122.2, 124.8, 125.6, 129.8, 131.7, 134.6, 147.8, 151.4, 155.7, 157.9 (aromatic carbons), pyrimidine nucleus: 113.8, 115.6, 164.1, OCH₃: 55.6.

4-(4-methoxyphenyl)-6-(2-nitrophenyl)pyrimidine-2-thiol (M10): M. weight: 339.37; M. Point (°C): 68–70; Rf: 0.76; % yield: 72; IR (cm^{–1}): 2940 (C–H stretch), 1608 (C=C stretch, –methoxyphenyl and nitrophenyl group), 728 (C–C stretch), 1679 (C=N stretch or N=CH stretch, pyrimidine ring), 1363 (C–N stretch, pyrimidine), 841 (C–N stretch), 645 (C–S stretch) 2602 (S–H stretch), ¹H-NMR DMSO-*d*₆, δ ppm: 7.07–8.05 (m, 8H, Ar–H), 8.93 (s, 1H, CH of pyrimidine ring), 3.43 (s, 1H, SH), 3.81, 3.83 (s, 3H, –OCH₃), ¹³C NMR: (122.4, 124.8, 125.7, 130.2, 131.8, 134.8, 150.2, 155.6, 157.0), pyrimidine nucleus: (113.2, 115.6, 163.5), OCH₃–(55.4, 56.2)

4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidine-2-thiol (M11): M. weight: 339.37; M. Point (°C): 86–88; Rf: 0.89; % yield: 68; IR (cm^{–1}): 3181 (C–H stretch), 666 (C–C stretch), 1650 (C=N stretch pyrimidine ring), 1218 (C–N stretch, pyrimidine), 1512 (N=O stretch) 818 (C–N stretch, Ar–NO₂), 1338 (N=O stretch, –NO₂), 1014 (C–O–C stretch, –OCH₃), 2826 (C–H stretch, R–CH₃); ¹H-NMR DMSO-*d*₆, δ ppm: 7.18–8.32 (m, 8H, Ar–H), 8.0 (s, 1H, CH of pyrimidine ring), 3.35 (s, 1H, SH), 3.89 (s, 3H, –OCH₃). ¹³C NMR: 122.1, 124.6, 125.8, 129.6, 131.9, 134.5, 147.6, 150.8, 155.9, 157.3 (aromatic carbons), pyrimidine nucleus: 113.6, 115.8, 164.0, OCH₃: 55.4

4-(4-methoxyphenyl)-6-(3-nitrophenyl)pyrimidine-2-thiol (M12): M. weight: 339.37; M. Point (°C): 74–76; Rf: 0.85; % yield: 48; IR (cm^{–1}): 1332, 1513 (–NO₂), 1516 (C–H bend.) 2978 (C–H stretch), 1651 (C=C stretch), 726 (C–C stretch), 1613 (C–N stretch, pyrimidine), 2876 (S–H stretch), 1175 (C–O–C stretch, –OCH₃), 674 (C–N stretch); ¹H-NMR DMSO-*d*₆, δ ppm: 7.07–8.37 (m, 8H, Ar–H), 8.94 (s, 1H, CH of pyrimidine ring), 3.43 (s, 1H, SH), 3.82 (s, 3H, –OCH₃); ¹³C NMR: 122.3, 124.6, 125.4, 128.9, 130.5, 132.6, 135.6, 147.1, 151.4, 155.8 (aromatic carbons), pyrimidine nucleus: 113.6, 115.7, 163.5, OCH₃: 55.4.

4-(4-aminophenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M13): M. Weight: 309.09; M. Point (°C): 140–142; Rf: 0.72; % yield: 82; IR (cm^{–1}): 3617 (N–H stretch –NH₂), 1516 (C–H bend.) 3007 (C–H stretch), 1617 (C=C stretch), 748 (C–C stretch), 1617 (C–N stretch, pyrimidine), 2726 (S–H stretch) 1174 (C–O–C stretch, –OCH₃), 667 (C–N stretch); ¹H-NMR DMSO-*d*₆, δ ppm: 7.04–8.06 (m, 8H, Ar–H), 8.96 (s, 1H, CH of pyrimidine ring), 3.41 (s, 1H, SH), 3.74, 3.82 (s, 3H, –OCH₃); 4.85 (s, 2H, –NH₂); ¹³C NMR: 123.4, 124.4, 125.6, 130.4, 131.6, 134.6, 150.4, 155.3, 157.1 (aromatic carbons), pyrimidine nucleus: 113.1, 115.4, 163.3, OCH₃: 55.3, 56.5.

4-(4-(dimethylamino)phenyl)-6-(4-methoxyphenyl)pyrimidine-2-thiol (M14): M. weight: 337.12; M. Point (°C): 160–162; Rf: 0.65; % yield: 70; IR (cm^{–1}): 2940 (C–H stretch), 1511 (C=C stretch), 732 (C–S stretch–SH), 1602 (C=N stretch or N=CH stretch, pyrimidine ring), 1357 (C–N stretch, phenyl ring), 2940 (C–H stretch, R–CH₃), 1250 (C–N stretch –N(CH₃)₂), 1174 (C–O–C stretch, –OCH₃); ¹H-NMR DMSO-*d*₆, δ ppm: 7.05–8.07 (m, 8H, aromatic H), 8.98 (s, 1H, CH of pyrimidine ring), 3.42 (s, 1H, SH), 3.82, 3.84 (s, 3H, –OCH₃), 2.91 (s, 6H, N(CH₃)₂); ¹³C NMR: Aromatic carbons: (122.6, 124.7, 125.2, 130.7, 131.5, 133.8, 152.2, 155.7, 157.1), Pyrimidine nucleus: 113.4, 115.8, 163.7, Methoxy carbon (–OCH₃): 55.8, 56.3, dimethylamine carbons (NCH₃): 43.1

3.4. Molecular Docking Methodology:

The molecular docking analysis was performed to predict the binding mode and affinity of the synthesized pyrimidine derivatives by the GLIDE docking program of Maestro v11.5 (Schrodinger 2018-1). The three-dimensional crystal structure of dihydrofolate reductase (DHFR) (PDB ID: 2RGO, chain A) was retrieved from the Protein Data Bank (<https://www.rcsb.org/structure/2RGO>). Protein was prepared using the Protein Preparation Wizard in Maestro, and the water molecules beyond 5 Å from the ligand were removed. The missing chain and hydrogen were added. The protein structure was then optimized and minimized using the OPLS4 force field. The 2D structures of the synthesized compounds were drawn using ChemDraw 15.0 and imported into Maestro. The LigPrep tool was used to generate 3D conformations and protonation states at pH 7.0 ± 2.0, using the OPLS4 force field. Energy minimization was applied to generate low-energy conformers for docking. Docking was performed using Glide Standard Precision (XP) mode. The best-ranked poses based on GlideScore (kcal/mol) were selected and analysed. The docking protocol was validated by redocking the

native ligand into the active site. Post-docking minimization was enabled to refine poses. The docked complexes were visualized using Maestro Pose Viewer detailed interaction mapping ²².

3.5. In silico ADMET prediction:

The pharmacokinetic properties of the synthesized chalcones and their pyrimidine derivatives (C1-C14 & M1-M14) were predicted using the QikProp module available in the Schrödinger software package. Prior to ADME analysis, the three-dimensional structures of all compounds were prepared with LigPrep employing the OPLS4 force field, and appropriate protonation states were assigned at physiological pH (7.0 ± 2.0). The ADME parameters were then calculated using the default QikProp protocol ²².

3.6. Biological assay:

3.6.1. Antimicrobial assay:

Zone of inhibition

The antibacterial activity of the synthesized compounds (C1–C14 and M1–M14) was investigated against three Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, and *Enterococcus faecalis*), two Gram-negative bacteria (*Haemophilus influenzae* and *Klebsiella pneumoniae*) and fungal strain (*Candida albicans*) using the agar well diffusion method described by Balouiri et al. These microorganisms were selected because they are clinically important pathogens responsible for a wide range of community- and hospital-acquired infections. Their inclusion also enabled the evaluation of antibacterial activity against organisms possessing different cell wall architectures.

Sterile Mueller–Hinton agar was poured into Petri plates and allowed to solidify. The bacterial suspension was evenly spread over the agar surface using sterile cotton swabs. Wells of 6 mm diameter were punched aseptically, and 50 μ L of each test solution (10, 20, and 30 μ g/ml, prepared in DMSO) was introduced into the respective wells. Azithromycin (10 μ g/ml) served as the reference antibacterial drug, whereas DMSO was used as the negative control. Following incubation at 37 ± 0.5 °C for 24 h, the diameter of the inhibition zone surrounding each well was measured in millimetres ²³. Inhibition zones of 10 mm or greater were considered indicative of antibacterial activity, while smaller zones or the absence of a clear zone were recorded as 00.

Minimum Inhibitory concentration:

The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 125 μ g/ml. Two-fold serial dilutions were carried out in sterile Mueller–Hinton Broth using 96-well microtiter plates. Initially, 100 μ L of Mueller–Hinton Broth was added to each well. Subsequently, 100 μ L of the compound solution (125 μ g/ml) was transferred to the first well and mixed thoroughly. Thereafter, 100 μ L was transferred sequentially from the first well to the second well, from the second to the third, and so on, to obtain concentrations of 125, 62.5, 31.25, 15.625, 7.8125, and 3.90625 μ g/ml. After the final transfer, 100 μ L was discarded from the last well to maintain an equal volume in all wells. Finally, 100 μ L of the standardized microbial inoculum was added to each well, resulting in a final volume of 200 μ L per well ²⁴.

3.6.2. In vitro breastcancer assay (MDB-MB-231)

The in vitro cytotoxic activity of the synthesized compounds was evaluated using the Sulforhodamine B (SRB) assay in triplicate. Human cancer cell lines were cultured in the appropriate growth medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO₂, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. The synthesized compounds were dissolved in a suitable solvent to prepare a stock solution, followed by four serial 10-fold dilutions in complete culture medium. The diluted samples were added to the cells and incubated for 48 h. Cell growth was terminated by fixing the cells with cold 10% trichloroacetic acid (TCA), followed by staining with 0.4% Sulforhodamine B (SRB). The bound dye was solubilized in 10 mM Tris base, and absorbance was measured at 540 nm using a microplate reader with a reference wavelength of 690 nm.

The percentage cell growth was calculated by comparing the absorbance of treated wells with untreated control wells. The GI₅₀ value, defined as the concentration of the compound required to inhibit 50% of cell growth, was determined from the dose–response data ²⁵.

3.6.3. Antioxidant assay:

DPPH Assay:

Free-radical scavenging activity of the chalcones and their 6-methoxyphenyl pyrimidine derivatives (M1-M14) and ascorbic acid as a reference was assessed by 2,2-diphenyl-1-picrylhydrazyl, i.e., DPPH scavenging assay ²². The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay relies on the colorimetric transformation of a purple methanol solution (DPPH•+HA) to a yellow hydrazine derivative (DPPH-H+A•), signifying antioxidant activity via hydrogen atom or electron donation. A stock solution of 100 mM DPPH in methanol was prepared and stored in the dark at 25°C for 3-4 hours before analysis. The radical was stable in this form for several hours when stored in the dark at 4 °C. The DPPH solution, 0.01 mM, was prepared in methanol using 0.003 g of DPPH dissolved in 100 ml of methanol and kept in the dark to protect it from light. Serial dilution of synthesized compounds and standard Ascorbic Acid (12.5, 25, 50, 75, 100 μ g/ml) was prepared in methanol. Then an equal volume of DPPH solution and test sample was mixed and incubated in the dark at room temperature for 30 minutes. Measure the decrease in absorbance of DPPH radicals at 517 nm. The same procedure was carried out for test samples, and Methanol was used as the blank. Then the percentage inhibition of DPPH radicals is calculated using the formula

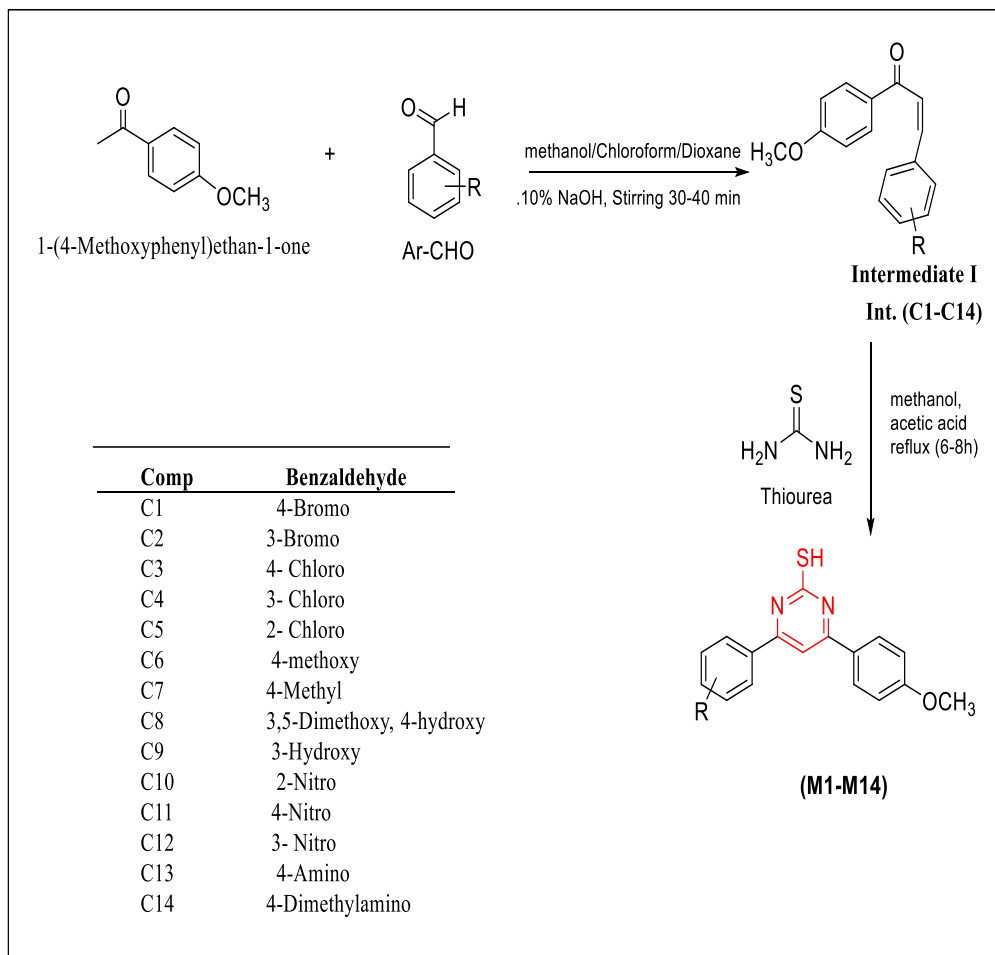
$$\% \text{ Inhibition} = \frac{\text{Absorbance in the control} - \text{Absorbance in the sample}}{\text{Absorbance in the control}} \times 100$$

A scatter plot was generated between concentration (μ g/ml) and percentage inhibition (%) for each compound ²³.

4. RESULTS AND DISCUSSION:

4.1. Chemistry:

The Pyrimidine derivatives (M1-M14) were synthesized via Claisen–Schmidt condensation between appropriately substituted using scheme 1. 4-methoxy acetophenones and substituted benzaldehydes under basic condition (NaOH 10 %). In the reaction enolate ion is formed and reaction followed by nucleophilic attack on the aldehyde and subsequent dehydration to afford (E)- α , β -unsaturated ketones (chalcones). In the second step, intermediates chalcones were further reacted to synthesize pyrimidine-substituted derivatives via a condensation with thiourea, in the presence of acetic acid. The reaction followed Michael's addition of the nucleophile to the α , β -unsaturated system, followed by cyclization and aromatization to yield substituted 2-thioxypyrimidines. The completion of the reaction was confirmed by TLC.



Scheme 1: 4-(4-methoxyphenyl)-6-(substituted)pyrimidine-2-thiol derivatives

4.2. Docking result

All synthesized compounds (M1–M14), along with the standard drug trimethoprim, were docked into the active site of the 2RGO protein. The docking results were analysed based on the docking score and glide energy obtained from GLIDE. The docking scores and Glide energies are presented in Table 1 and Fig. 2. Compounds M5, M12, M10, and M4 exhibited the most favourable docking scores, suggesting strong binding affinity compared to the standard.

The standard drug trimethoprim showed a docking score of -8.1087 , which was significantly lower than many of the tested compounds. This suggests that several synthesized derivatives, particularly Compound M5 exhibited the strongest binding affinity (-10.05 kcal/mol) due to formation of hydrogen bonding with Asp27 and Ile94 along with hydrophobic interactions involving Leu28, Phe31 and Tyr100, stabilizing the ligand within the GlpO active pocket.

Further the compounds were evaluated for their breast cancer (cell line MDB-MB-231) potential through molecular docking studies using Adriamycin as the reference drug. Adriamycin showed the highest binding affinity with a docking score of -9.177 and a Glide energy of -58.22 kcal/mol. Among the synthesized derivatives, M4 exhibited the best docking score (-7.357), while M6 showed the most favourable Glide energy (-55.29 kcal/mol), indicating the formation of a stable ligand–protein complex. M12 also demonstrated good binding affinity, whereas M2 and M1 showed comparatively weaker interactions as shown in Table 2.

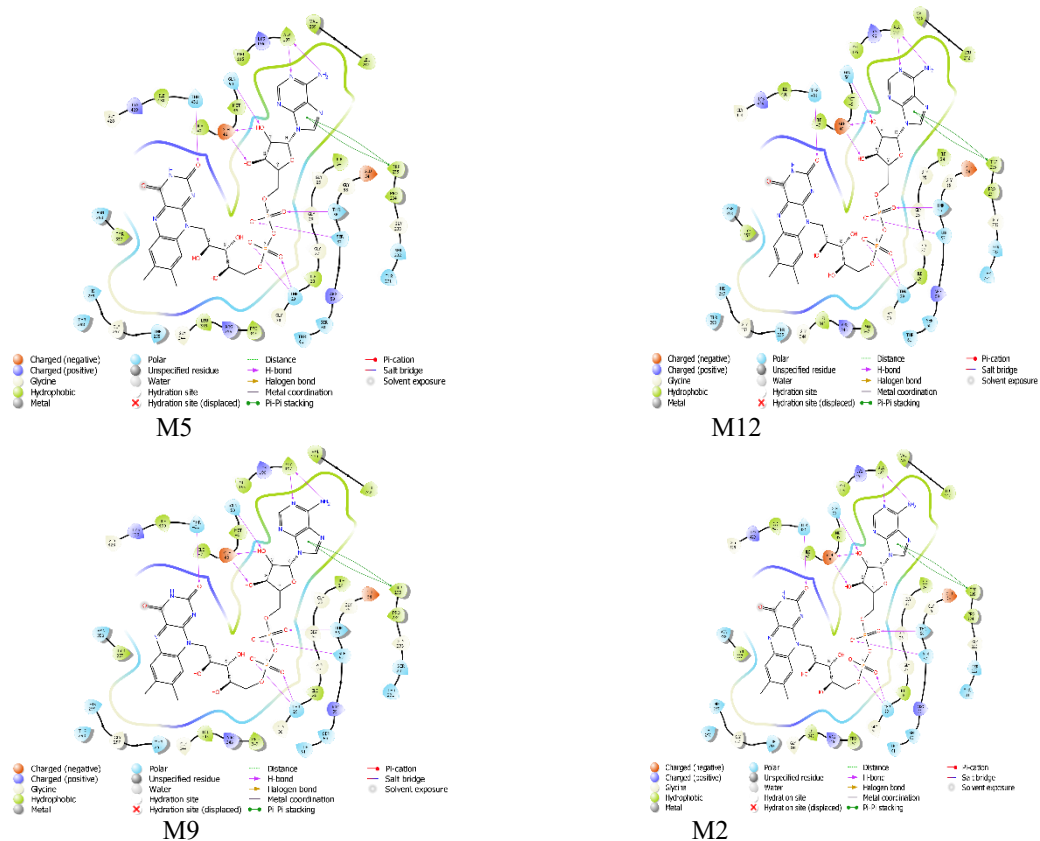


Fig. 2.: 2D interaction diagram of most active compounds as antimicrobial

Table 1: Docking score of synthesized Chalcones (C1-C14) and Pyrimidine derivatives (M1-M14) on PDB id: 2RGO

Compounds	Docking Score	Glide Energy	Compounds	Docking Score	Glide Energy
C13	-10.01229	-38.311	M5	-10.0513	-50.2340989
C1	-8.419324	-38.493	M12	-9.82761	-53.2503839
C12	-8.12048	-42.487	M10	-9.80705	-50.2060609
C7	-7.99314	-35.8613	M4	-9.79738	-51.4055057
C10	-7.89697	-37.802	M9	-9.74129	-52.2366662
C4	-7.81887	-44.1699	M2	-9.70808	-52.1957912
C8	-7.65056	-43.9245	M8	-7.89347	-52.3520374
C6	-7.65056	-43.9245	M1	-7.83286	-45.9398804
C14	-7.54745	-46.7563	M11	-7.56929	-46.5864782
C3	-7.29039	-46.0508	M14	-7.41359	-46.8323236
C2	-7.26962	-47.242	M6	-7.33831	-46.0996066
C9	-7.02936	-44.0015	M7	-7.28105	-45.4734688
C11	-7.02244	-45.4867	M3	-6.91924	-45.2979417
C5	-6.80545	-40.4403	M13	-6.91607	-50.0862343
Trimethoprim	-8.10868	-39.6096			

Table 2: Docking score of synthesized most active Pyrimidine derivatives as breast cancer (MDB-MB-231) PDB id: 5GWK

Compounds	Docking score	Glide energy
adriamycin	-9.17713	-58.22118568
M4.	-7.35738	-46.71421599
M6.	-7.11897	-55.28848267
M12.	-7.11377	-51.38252068
M2.	-6.81529	-44.33552408
M1.	-6.66208	-45.50953954

4.3. ADMET analysis:

The ADMET properties of the synthesized compounds (C1–C14 and M1–M14) were predicted using the Schrödinger QikProp module to evaluate their drug-likeness and pharmacokinetic characteristics. All newly synthesized compounds exhibited molecular weights between 252.31 and 373.26 g/mol, which are within the acceptable range for orally active drug candidates. All compounds showed zero or one Lipinski's Rule of Five (RO5) violation, indicating favourable drug-like properties.

The hydrogen bond donor (HBD) values ranged from 0.0 to 3.0, while hydrogen bond acceptor (HBA) values varied from 2.75 to 5.50, showed appropriate balance between hydrogen-bonding ability and membrane permeability. Predicted Caco-2 cell permeability (QPPCaco) values ranged from 554.67 to 6781.78 nm/s, indicating good to excellent intestinal permeability for most compounds. In particular, compounds M1, M2, M3, M4, M5, M6, M7, and M14 exhibited Most of the synthesized derivatives exhibited very high predicted Caco-2 permeability, indicating good potential for intestinal membrane transport. The calculated QPlogPo/w values (2.92–5.33) reflected moderate to high lipophilicity, suggesting favourable membrane permeability. Predicted aqueous solubility (QPlogS) values ranged from –3.52 to –6.17, with the majority of the compounds displaying acceptable solubility characteristics. In addition, all derivatives showed excellent predicted human oral absorption, with most compounds exhibiting 100% absorption. Compounds C11 (94.23%), C12 (95.38%), and M8 (93.34%) also demonstrated high predicted oral absorption, supporting their favourable oral bioavailability as shown in Table 3.

The QikProp analysis demonstrated that the most of the synthesized pyrimidine derivatives possess desirable pharmacokinetic properties, including favourable permeability, acceptable lipophilicity, high predicted oral absorption, and compliance with Lipinski's Rule of Five. These findings support their potential as promising drug-like candidates for further biological evaluation.

Table 3. In silico ADME and drug-likeness properties of synthesized compounds

Comp.	Mol. Wt. (g/mol)	HBD	HBA	QPP Caco	QPlog Po/w	QLogS	Oral Absorption (%)	RO5 Violations
C1	317.181	0	2.75	5224.047	4.289	-4.491	100	0
C2	317.181	0	2.75	5256.522	4.288	-4.477	100	0
C3	272.73	0	2.75	5220.977	4.183	-4.366	100	0
C4	272.73	0	2.75	5221.698	4.186	-4.367	100	0
C5	272.73	0	2.75	5353.483	4.134	-4.237	100	0
C6	268.312	0	3.5	5193.664	3.766	-3.76	100	0
C7	252.312	0	2.75	5211.343	4.02	-4.212	100	0
C8	314.337	1	5	1979.465	3.318	-3.859	100	0
C9	254.285	1	3.5	1583.698	3.093	-3.894	100	0
C10	283.283	0	3.75	861.322	3.029	-3.52	100	0
C11	283.283	0	3.75	622.503	2.95	-3.641	94.226	0
C12	283.283	0	3.75	701.466	2.988	-3.574	95.376	0
C13	253.3	1.5	3.75	1352.402	2.917	-3.606	100	0
C14	281.354	0	3.75	4852.076	4.087	-4.383	100	0
M1	373.26	0.8	2.75	6753.322	5.329	-6.144	100	1
M2	373.26	0.8	2.75	6780.568	5.326	-6.127	100	1
M3	328.81	0.8	2.75	6753.923	5.252	-6.028	100	1
M4	328.81	0.8	2.75	6781.778	5.258	-6.037	100	1
M5	328.81	0.8	2.75	6681.694	5.157	-5.843	100	1
M6	324.39	0.8	3.5	6804.701	4.853	-5.504	100	0
M7	308.39	0.8	2.75	6730.134	5.068	-5.857	100	1
M8	370.42	3	5.5	554.667	2.952	-4.579	93.341	0
M9	310.37	1.8	5	2408.264	4.286	-5.468	100	0
M10	339.36	0.8	3.75	788.19	4.329	-6.008	100	0
M11	339.36	0.8	3.75	801.077	4.054	-5.44	100	0
M12	339.36	0.8	3.75	801.637	4.053	-5.439	100	0
M13	309.38	2.3	3.75	1788.927	3.767	-4.806	100	0
M14	337.43	0.8	3.75	6466.735	5.19	-6.166	100	1

4.4. Antimicrobial result:

Zone of inhibition

The antimicrobial activity of the synthesized chalcone (C1–C14) and pyrimidine (M1–M14) derivatives was examined against three Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, and *Enterococcus faecalis*), two Gram-negative bacteria (*Haemophilus influenzae* and *Klebsiella pneumoniae*), and the fungal strain *Candida albicans* using the

agar well diffusion method. The inhibitory effect of each compound was assessed by measuring the zone of inhibition (ZOI) at concentrations of 10, 20, and 30 µg/ml. The corresponding results are summarized in Tables 4 and 5.

The most of the synthesized compounds inhibited microbial growth in a concentration-dependent manner. As the concentration increased, the inhibition zones generally became larger, indicating improved antimicrobial activity. Some compounds produced no visible inhibition at the lowest concentration against selected microorganisms but became active at higher concentrations.

Among the Gram-positive bacteria, the chalcone derivatives exhibited varying levels of activity. Compound C9 produced the largest inhibition zones against *Staphylococcus aureus* and *Micrococcus luteus* (22 mm at 30 µg/ml). Compound C14 was effective against *Micrococcus luteus* and *Enterococcus faecalis*, while C11 recorded the highest inhibition against *Enterococcus faecalis* (21 mm at 30 µg/ml). Compounds C1, C2, C3, C7, and C13 also displayed consistent antibacterial activity across the tested concentrations.

The pyrimidine derivatives also showed good antibacterial activity. Compound M5 exhibited the strongest inhibitory effect against *Staphylococcus aureus* with a maximum inhibition zone of 22 mm at 30 µg/ml and maintained good activity against the remaining Gram-positive strains. M9 and M14 were also active against all three Gram-positive bacteria, whereas M1, M4, M12, and M13 produced moderate inhibition. In contrast, M6, M10, and M11 displayed lower activity under identical experimental conditions.

A comparable dose-dependent response was also observed against the Gram-negative bacterial strains; however, the inhibition zones were generally lower than those obtained for the Gram-positive organisms. Among the chalcone derivatives, compound C8 showed the strongest activity against *Haemophilus influenzae*, producing an inhibition zone of 22 mm at 30 µg/ml. In the case of *Klebsiella pneumoniae*, M5 displayed the greatest antibacterial effect with a zone of inhibition of 19 mm, followed by C14 (18 mm). Moderate activity against one or both Gram-negative bacteria was also observed for compounds C1, C9, C12, M1, M8, and M14.

The antifungal activity against *Candida albicans* followed the same concentration-dependent pattern. Chalcone derivatives C8 and C11 produced the largest inhibition zones (23 mm at 30 µg/ml), followed by C10, C12, and C14. Within the pyrimidine series, M5 and M14 exhibited the highest antifungal activity (22 mm), whereas M10, M11, and M12 also showed considerable growth inhibition. The results indicate that both chalcone and pyrimidine derivatives possess broad-spectrum antimicrobial activity. Several compounds remained active against Gram-positive bacteria, Gram-negative bacteria, and *Candida albicans*, with M5 among the pyrimidine derivatives and C8 and C9 among the chalcone derivatives emerging as the most active members of their respective series.

Table 4: Antibacterial activity of synthesized compounds (C1-C14 & M1-M14): ZOI against Gram-positive bacteria

Comp	Observed inhibition zone (mm) Gram-positive bacteria											
	S. aureus				M. luteus				E. faecalis			
	(µg/ml)											
	10	20	30	+ve	10	20	30	+ve	10	20	30	+ve
C1	12	15	19	25	13	17	20	22	12	15	18	22
C2	14	17	20	24	13	16	18	23	13	16	19	23
C3	13	17	20	23	00	15	19	23	12	16	20	26
C4	12	15	17	22	10	13	16	26	00	14	17	24
C5	10	14	16	22	12	16	19	22	00	13	15	26
C6	00	15	17	23	11	14	16	24	12	15	17	21
C7	13	16	19	23	12	15	18	21	13	16	19	23
C8	14	15	17	24	14	15	17	22	14	14	15	25
C9	13	17	22	22	13	17	22	23	11	15	19	26
C10	13	14	15	23	13	14	14	25	13	15	16	24
C11	12	14	16	25	12	16	21	26	13	13	14	23
C12	00	15	18	23	00	13	17	23	00	14	18	27
C13	00	15	19	24	12	16	20	24	10	14	17	26
C14	12	15	19	26	13	18	22	25	12	14	20	24
M1	11	13	19	23	00	13	19	24	12	14	21	23
M2	00	14	17	24	13	15	18	24	11	13	20	20

M3	00	12	15	24	00	15	20	24	11	14	16	25
M4	00	16	19	23	12	14	16	25	17	18	20	24
M5	16	18	22	23	11	13	20	24	14	15	16	23
M6	12	13	14	22	11	12	14	26	14	15	16	23
M7	00	12	16	23	00	13	18	22	10	13	17	21
M8	13	14	16	25	13	14	14	21	13	13	14	23
M9	12	16	21	21	11	15	20	20	10	14	18	20
M10	12	12	13	21	12	13	13	23	12	14	15	27
M11	11	12	13	21	10	11	12	22	12	12	13	22
M12	10	13	17	22	11	14	18	25	10	12	16	20
M13	10	14	18	22	11	15	19	23	00	13	17	21
M14	11	15	19	23	12	17	21	22	11	15	19	21
DMSO	-	-	-	-	-	-	-	-	-	-	-	-

+ve= azithromycin 10µg/ml

Table 5: Antimicrobial activity of synthesized compounds (C1-C14 & M1-M14): ZOI against Gram-positive bacteria and fungal strain

Compounds	Observed inhibition zone (mm)											
	Gram-negative bacteria								Fungal strain			
	H. influenza				K. pneumonia				C. albicans			
	10	20	30	+ve	10	20	30	+ve	10	20	30	+ve
C1	13	16	19	24	11	15	17	26	11	15	19	22
C2	00	11	14	21	00	13	16	25	00	12	16	21
C3	00	12	15	23	10	14	17	26	12	15	20	23
C4	00	10	12	22	00	12	15	24	10	14	18	21
C5	00	12	14	26	00	11	13	27	00	13	18	22
C6	00	10	12	25	10	13	15	21	11	13	14	20
C7	00	12	15	24	11	14	17	23	00	12	16	22
C8	18	20	22	23	14	14	14	24	17	22	23	23
C9	00	13	16	23	11	15	17	26	00	12	16	25
C10	11	13	15	25	11	12	13	28	16	19	23	25
C11	13	15	16	23	11	12	15	23	17	22	23	24
C12	00	13	17	24	00	12	16	26	15	19	22	24
C13	00	13	17	22	00	12	16	24	11	15	18	21
C14	11	15	19	23	00	14	18	23	14	16	20	23
M1	00	12	17	23	13	15	17	21	12	15	18	20
M2	00	13	18	23	00	11	16	29	11	14	16	21
M3	00	11	21	22	11	14	16	21	11	13	16	20
M4	00	13	15	23	13	16	17	23	13	16	20	24
M5	10	14	21	21	13	15	19	22	14	18	22	25
M6	14	14	15	22	12	13	14	21	13	16	19	23
M7	00	11	14	29	00	12	15	28	11	14	16	20
M8	17	19	22	28	13	13	13	21	12	15	18	21
M9	00	12	15	20	10	14	16	20	12	15	17	22
M10	10	12	14	21	10	11	12	23	13	18	21	23
M11	12	14	15	26	10	11	14	23	14	18	21	21
M12	00	11	15	21	00	10	14	21	13	17	21	23
M13	00	12	16	21	00	11	15	20	12	16	19	21
M14	10	14	18	21	00	13	17	20	15	19	22	23
DMSO	-	-	-	-	-	-	-	-	-	-	-	-

+ve= azithromycin 10µg/ml, Fluconazole:10µg/ml,



Fig.3.: Antimicrobial activity (ZOI) on Gram positive and Gram-negative in bacteria of M1&M5

MIC (Minimum inhibitory concentration (MIC) studies)

The antimicrobial potential of the selected pyrimidine derivatives was further evaluated by determining their minimum inhibitory concentration (MIC) against five bacterial strains (*Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Haemophilus influenzae*, and *Klebsiella pneumoniae*) and one fungal strain (*Candida albicans*).

Among the tested compounds, MIC values ranged from 7.8125 to 31.25 µg/ml, indicating variable antimicrobial potency against the investigated microorganisms. Compounds C9 and M5 exhibited the broadest antibacterial activity, producing MIC values of 7.8125 µg/ml against *S. aureus*. In addition, M5 showed the same MIC against *H. influenzae* and *K. pneumoniae*, while C9 demonstrated comparable activity against *M. luteus*. Compound C11 displayed its highest activity against *E. faecalis* with an MIC of 7.8125 µg/ml. Similarly, C8 and M3 were most active against *H. influenzae*, whereas M14 and C14 showed improved activity against *M. luteus*, each with an MIC of 7.8125 µg/ml.

Most of the remaining bacterial isolates were inhibited at MIC values of 15.625 µg/ml, while a few compound–organism combinations required 31.25 µg/ml, indicating comparatively lower antibacterial activity. Overall, compounds C9, M5, C11, and M3 demonstrated the most promising antimicrobial profiles among the synthesized derivatives.

The antifungal evaluation against *Candida albicans* revealed MIC values ranging from 7.8125 to 31.25 µg/ml. Compounds C9 and M3 exhibited the highest antifungal activity with MIC values of 7.8125 µg/ml, whereas the remaining compounds inhibited fungal growth at 15.625 or 31.25 µg/ml²⁷.

The reference drugs showed superior antimicrobial activity, with Azithromycin producing MIC values of 1.95–3.90 µg/ml against the bacterial strains, while Fluconazole exhibited an MIC of 7.8125 µg/ml against *C. albicans* (Table 6).

Table 6: Antimicrobial activity (MIC) of selected compounds

Compounds	<i>S. aureus</i>	<i>M. luteus</i>	<i>E. faecalis</i>	<i>H. influenzae</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
C8	15.625	31.25	31.25	7.8125	15.625	15.625
C9	7.8125	7.8125	15.625	15.625	15.625	7.8125
C11	15.625	15.625	7.8125	15.625	15.625	15.625
C14	15.625	7.8125	15.625	15.625	31.25	31.25
M3	15.625	15.625	15.625	7.8125	15.625	7.8125
M5	7.8125	15.625	15.625	7.8125	7.8125	15.625
M9	7.8125	15.625	15.625	15.625	15.625	15.625
M14	15.625	7.8125	15.625	15.625	15.625	15.625
Azithromycin	1.95	1.95	3.90	1.95	3.90	-
Fluconazole	-	-	-	-	-	7.8125

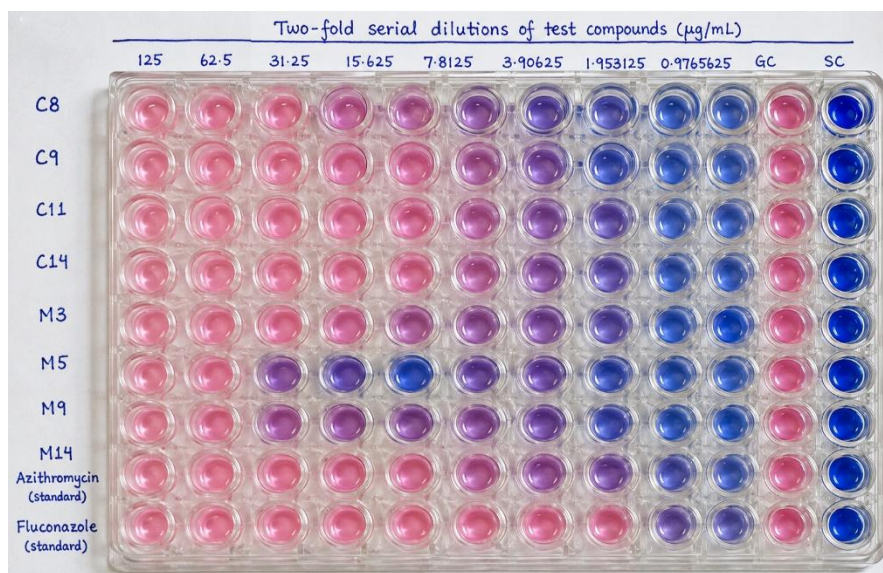


Figure 4: Minimum Inhibitory Concentration (MIC) of Selected Compounds (µg/ml)

4.5. In vitro anticancer activity result

Based on the molecular docking study, selected compounds M1, M2, M4, M6, and M12 exhibiting favorable binding affinity towards the selected anticancer target were selected for in vitro anticancer evaluation against the MDA-MB-231 human breast cancer cell line.

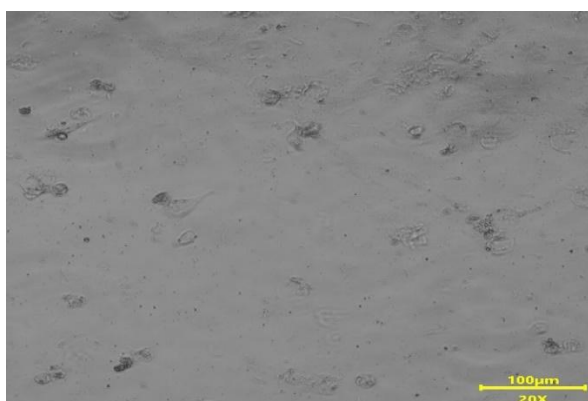
The antiproliferative activity of the selected compounds was evaluated at four molar concentrations (10^{-7} – 10^{-4} M), and the percentage control growth was determined. Among the tested compounds, M4 showed the highest antiproliferative activity, reducing the percentage control growth to –34.6% at 10^{-4} M, indicating pronounced cytotoxicity. M1 also demonstrated significant growth inhibition, with only 4.4% control growth at the highest concentration tested. In contrast, M2, M6, and M12 exhibited comparatively weaker activity, with percentage control growth values of 50.3%, 52.6%, and 55.8%, respectively, at 10^{-4} M.

The calculated GI_{50} values further supported these observations. Compound M4 displayed the highest potency with a GI_{50} value of 5 µM, followed by M1 with a GI_{50} value of 8 µM. However, M2, M6, and M12 showed GI_{50} values greater than 100 µM, indicating weak growth inhibitory activity against the MDA-MB-231 cell line within the tested concentration range.

Adriamycin, used as the positive control, exhibited marked anticancer activity with a GI_{50} value of <0.1 µM and negative percentage control growth values at all tested concentrations, confirming its potent cytotoxic effect against MDA-MB-231 cells (Table 7).

Table 7: Anticytotoxicity of selected compounds against human breast cancer cell line MDB-MB-231

Human Breast Cancer Cell Line MDA-MB-231					
% Control Growth					
Molar Drug Concentrations					
Average Values					
Compounds	10-7M	10-6M	10-5M	10-4M	GI_{50} (µM)
M1	80.5	85.9	68.2	4.4	8
M2	86.6	93.1	92.7	50.3	>100
M4	85.2	96.0	88.0	-34.6	5
M6	83.8	95.2	91.5	52.6	>100
M12	81.7	85.1	82.3	55.8	>100
Adriamycin	-8.5	-8.4	-9.2	-16.8	<0.1



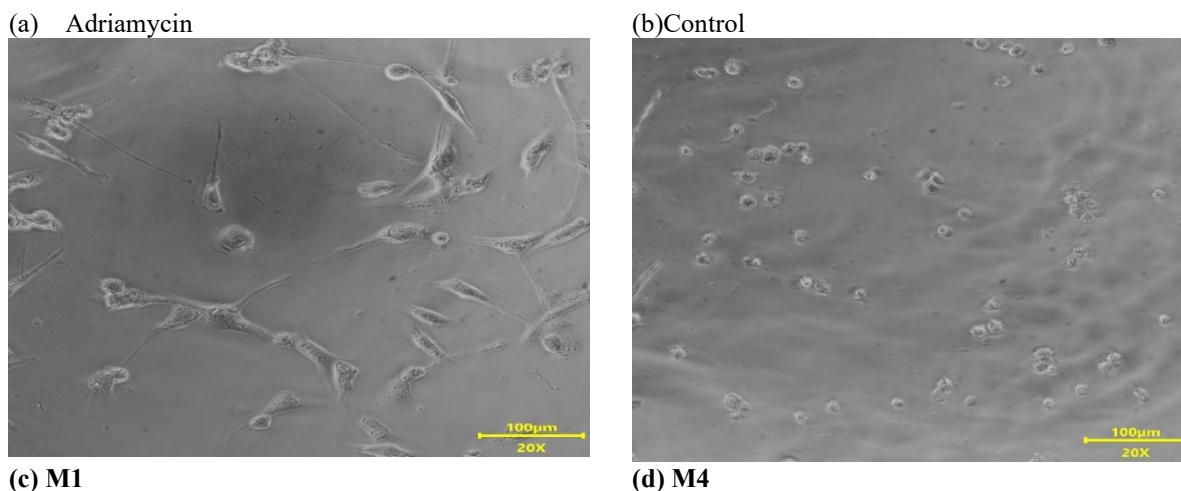


Figure 5: Anticytotoxicity of standard and most active compounds against human breast cancer cell line MDB-MB-231

4.5. Antioxidant result: Synthesized pyrimidine derivatives (M1-M15) were evaluated for their antioxidant potential using the DPPH radical scavenging assay at different concentrations (12.5, 25, 50, 75, and 100 µg/ml), and compared with standard ascorbic acid. Results of DPPH assay revealed that the percentage of radical scavenging activity (% RSA) increased with an increase in concentration of the tested compounds. Among the synthesized compounds, M8 and M6 exhibited excellent antioxidant potential with IC_{50} values of 26.23 µg/ml and 28.96 µg/ml, respectively, when comparable to the standard ascorbic acid exhibiting the highest scavenging activity with a % RSA of 91.57% at 100 µg/ml and an IC_{50} value of 28.69 µg/ml. Other compounds, such as M13 (IC_{50} = 44.35 µg/ml), also exhibited significant radical scavenging activity, indicating the effect of electron-donating substituents like hydroxyl and methoxy groups on antioxidant potential and compound M7 demonstrated lower antioxidant activity, with IC_{50} values above 90 µg/ml, displayed weaker DPPH scavenging potential as shown in Table 8 and Fig. 4²⁶⁻²⁷.

Among the chalcone derivatives, C8 showed the best antioxidant activity with an IC_{50} value of 48.38 µg/ml, followed by C14 (51.41 µg/ml), C6 (62.83 µg/ml), C7 (64.32 µg/ml), and C13 (64.33 µg/ml). The remaining chalcones exhibited comparatively lower free radical scavenging activity, with IC_{50} values above 70 µg/ml.

Table 8: Antioxidant activity of synthesized compounds (M1-M14) by DPPH assay

Compounds	% Inhibition RSA					IC ₅₀
	µg/ml					
	12.5	25	50	75	100	
C1	11.5	19.62	30.5	41.62	51.75	94.5074
C2	12.75	23.12	34.25	44.25	57	85.105
C3	14.37	22	33	43.87	54.75	88.6594
C4	14.37	23.12	33.87	44.75	55.5	86.7224
C5	12.12	18.87	29.62	41	51.75	95.5237
C6	16.62	30.87	45	58.75	68.87	62.8278
C7	14.37	27.37	47.37	59.25	66.5	64.3242
C8	19	37.12	55.5	72.5	80	48.3804
C9	13.12	23.5	37	52.75	67.12	71.1176
C10	12.37	16.25	27.25	36.75	41.87	118.059
C11	13.62	19.75	32.25	41.5	50.5	96.2651
C12	10.62	19.37	32.12	42	50.37	95.0812
C13	18.25	32.25	44.87	58.25	65.37	64.3322
C14	22.75	39.25	53.12	64.5	73.37	51.414
M1	23.68	35.08	49.12	64.0309	74.564	53.7352
M2	22.80	33.33	47.36	61.4051	71.9282	55.1465
M3	21.92	32.45	45.61	59.6412	69.2925	57.7901
M4	28.50	43.68	54.91	56.4923	61.1435	50.3571
M5	13.07	29.73	33.85	55.0872	81.5795	69.153
M6	43.15	50.43	56.92	65.0872	89.564	28.9614
M7	18.77	24.91	32.98	39.7384	48.3333	104.191

M8	39.29	54.82	60.961	72.287	88.7793	26.2268
M9	23.42	24.56	36.31	46.1435	64.2153	85.9694
M10	13.94	27.80	37.36	49.6412	59.564	73.9975
M11	17.71	25.61	39.56	48.5949	58.2461	75.1743
M12	19.12	28.68	40.78	53.1589	59.564	68.0678
M13	24.29	37.71	57.63	69.6412	83.7793	44.3413
M14	26.22	40.08	57.63	72.713	77.8002	44.241
Ascorbic Acid	37.01	49.8256	65.2616	79.4768	91.5795	28.6968

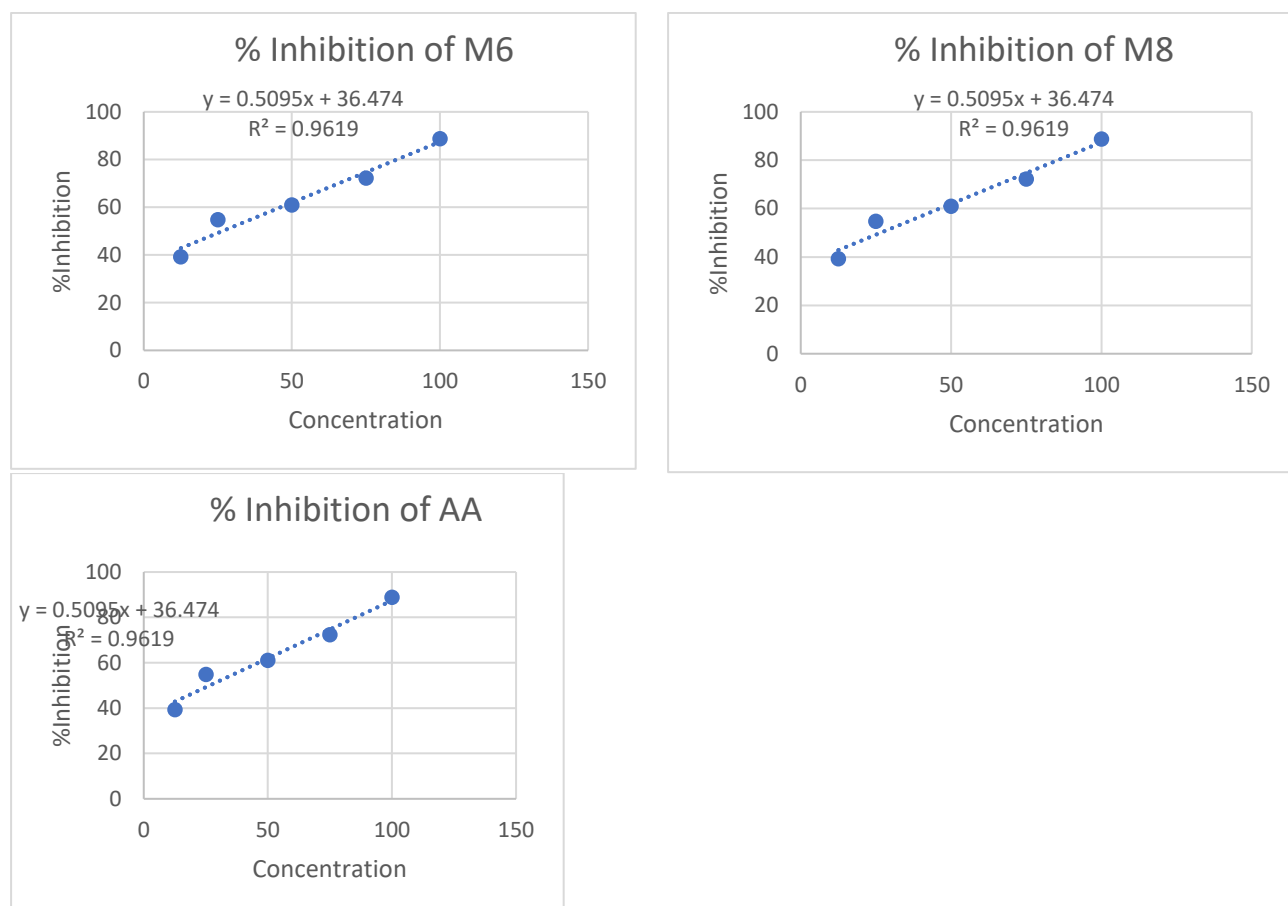


Fig. 4.: Graph represent %inhibition vs concentration of Ascorbic and M6, M8

5. Structure-activity relationship:

A structure–activity relationship (SAR) investigation was performed to establish correlations between molecular structure and biological evaluation shown in Fig. 5.

1. Compounds with EWG (-Cl, Br, NO₂) at the phenyl ring significantly enhanced antibacterial activity, particularly against *S. aureus*, *H. influenzae*, and *K. pneumoniae*.

- Compounds M5 (-Cl) exhibited superior antibacterial activity, producing larger zones of inhibition and lower MIC values against the tested microorganisms.
- Nitro-substituted derivatives (M10–M12) showed excellent docking scores against alpha-glycerophosphate oxidase, indicating favourable binding affinity; however, there in vitro antibacterial activity was comparatively moderate. These findings suggest that electron-withdrawing groups improve target binding, although additional physicochemical factors influence biological activity.
- These derivatives also demonstrated stronger binding affinities. H-bonding and π - π stacking (e.g., with Asp81, Arg84, Phe103), supporting the experimental potency.

2. Compounds with EDG (OH, OCH₃) exhibit moderate antibacterial but excellent antioxidant activity due to enhanced hydrogen-donating ability.

- M6 and M8 (multiple -OCH₃/-OH groups) exhibited lowest IC₅₀ values in DPPH assays, confirming the role of EDGs in radical scavenging.
- However, excessive polarity/bulk reduced membrane penetration in some strains, slightly compromising antibacterial potency.

3. Substituent position and planarity

- Para-substituted derivatives generally exceeded meta>ortho analogues, suggesting improved planarity and conjugation, which favours binding and cell penetration.
- Ortho substitution (e.g., sterically demanding groups) occasionally reduced activity, likely due to non-optimal binding orientation.

4. Role of the pyrimidine-2-thiol core

- The pyrimidine-2-thiol moiety plays an important role in biological activity by facilitating hydrogen-bonding interactions and stabilizing ligand–protein binding within the active site.
- Compounds maintaining extended conjugation between phenyl–pyrimidine rings (e.g., M5, M14) showed broad-spectrum antibacterial activity.

5. Lipophilicity–activity balance

- Optimal activity was observed for compounds with QPlogP $\approx$ 4.0–5.3 and PSA < 75 Å², ensuring a balance between membrane permeability and solubility.
- Highly lipophilic halogenated derivatives favoured antibacterial action, whereas moderately polar derivatives favoured antioxidant effects.

6. Anticancer activity:

- Compound M4 exhibited the strongest cytotoxic activity (GI₅₀ = 5 μM), which was consistent with its favourable docking score against the breast cancer target, suggesting that the substitution pattern of M4 promotes stable protein–ligand interactions and contributes to its antiproliferative activity.

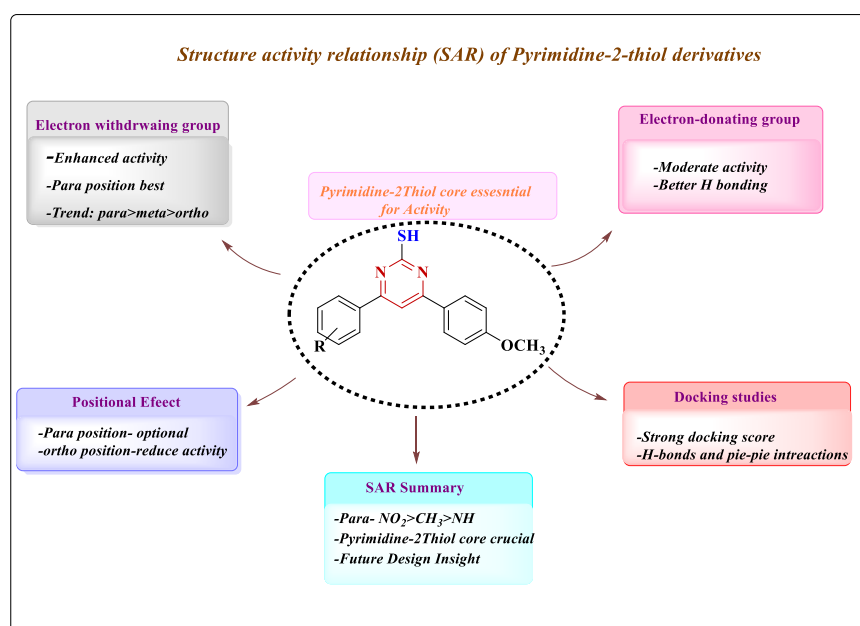


Fig. 5: Structure-activity relationship for the antibacterial and antioxidant activities of synthesized pyrimidine-2-thiol molecules

CONCLUSION:

In this study, a new class of chalcone pyrimidine derivatives was synthesized with appreciable yield. All the synthesized compounds were investigated for their in vitro antimicrobial, anticancer and antioxidant potential. It is evident that synthesized compounds M1, M5, and M14 exhibited notable antibacterial activity, whereas M6 and M8 demonstrated excellent antioxidant potential and compound M1 and M4 exhibited good breast cancer activity. The above synthesized compounds exhibited a marked enhancement in antimicrobial activity compared to earlier reported compounds. The Pyrimidine-2-thiol core molecules reported have the potential to serve as a promising lead scaffold with great potential to be used as antimicrobial, anticancer and antioxidant agents and as promising candidates for further efficacy evaluation.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

Minakshi .: Conceptualization, methodology, investigation, data curation, formal analysis, writing—original draft, and visualization. Mahesh Kumar: Supervision, conceptualization, methodology, review and editing, and project administration. Both the authors read and approved the final manuscript.

Data Availability

The data generated and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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