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DESIGN, SYNTHESIS, AND ANTIBACTERIAL EVALUATION OF NOVEL CURCUMIN ANALOGUES VIA MICROWAVE-ASSISTED CONDENSATION

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Keywords:

Curcumin analogues, Microwave-assisted synthesis, Biginelli reaction, Antibacterial activity, MIC

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ABSTRACT: Curcumin, the principal bioactive component of turmeric, is well-documented for its broad spectrum of pharmacological properties, including antioxidant and antibacterial activities. Nevertheless, its therapeutic use is restricted due to low solubility and limited bioavailability. In this study, eight novel curcumin derivatives (1A–8A) were synthesized using a microwave-assisted organic synthesis (MAOS) approach to enhance pharmacological efficacy. The synthetic strategy involved initial acetylation of curcumin followed by Biginelli-type cyclocondensation to incorporate heterocycles. All synthesized analogues were characterized using FT-IR, ^{1}H-NMR, and LC-MS. Antibacterial activity was assessed against *Escherichia coli* via broth dilution methods, and minimum inhibitory concentrations (MICs) were determined. Notably, Compounds 4A (9 micrograms per milliliter), 2A (10 micrograms per milliliter), and 8A (11 micrograms permilliter) displayed superior antibacterial activity compared to both curcumin (14 µg/mL) and the standard antibiotic ampicillin (16 µg/mL). These results indicate that strategic structural modifications to the curcumin scaffold, particularly through heterocyclic incorporation, can yield derivatives with significantly enhanced antibacterial potential. This work highlights the promise of curcumin analogues as potent antibacterial agents and supports further pharmacological investigation.

INTRODUCTION: Turmeric (*Curcuma longa L.*), commonly found in Asian cooking, is valued not only for its flavor-enhancing qualities but also for its broad medicinal benefits. Historically, it has served as a natural colorant and preservative, and has been traditionally used to manage health issues such as digestive problems, diarrhea, and liver-related disorders. The key active compound in turmeric is curcumin a yellow-colored polyphenol derived from the rhizome of the Zingiberaceae plant family.

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Curcumin has been the focus of numerous studies due to its wide range of biological effects, these investigations have highlighted its potent properties, particularly its antioxidant and anti-inflammatory actions [1, 2], anticancer [5], and antimicrobial effects [6, 10]. Its antioxidant potential is largely attributed to the phenolic and β-diketone functional groups, which facilitate radical scavenging and lipid peroxidation inhibition.

In addition to its antioxidant activity [7], curcumin has shown promising therapeutic roles in antiviral, antiulcer, anticarcinogenic [3], immunomodulatory, and other health-related applications [4]. Among these, its antibacterial properties have drawn particular interest. However, curcumin's poor solubility, low bioavailability, and chemical instability limit its direct therapeutic application.

To overcome these limitations and enhance antibacterial potency, structural modifications have been pursued. In particular, eliminating the β-diketone group and introducing heterocyclic structures have been found to enhance molecular stability and boost biological efficacy by strengthening interactions with microbial targets.

In this study, eight curcumin analogues **Table 1** meeting comprehensive screening criteria were synthesized. The synthetic approach involved acetylation of the phenolic hydroxyl groups, followed by Biginelli cyclocondensation reactions using substituted aldehydes, urea or thiourea, and β-ketoesters to generate heterocyclic scaffolds. This method facilitated the formation of dihydropyrimidinone-based structures, known for their broad pharmacological activities. Furthermore, the synthesis was conducted using Microwave-Assisted Organic Synthesis (MAOS), a powerful alternative to conventional techniques. MAOS significantly reduces reaction time and temperature while improving product yield, making it an efficient and sustainable method for the rapid generation of bioactive molecules. The curcumin analogues synthesized were subjected to in vitro antibacterial activity test.

MATERIAL AND METHODS:

Material: All reagents and chemicals used in this research were purchased from a reputable supplier ensuring analytical grade quality. The purity of the initial compounds was confirmed by measuring their melting or boiling points. Reaction monitoring was conducted using thin-layer chromatography. Melting points of the compounds were determined by employing Veego melting point device, and the observed temperatures were reported without any correction.

The progress and completion of the reaction were monitored through thin-layer chromatography performed on silica gel plates, using a solvent mixture of dichloromethane and methanol in a 5:1 ratio. The developed spots were visualized either under ultraviolet light or by exposing the plates to iodine vapors. The emergence of a new spot with a different Rf value, along with the fading or disappearance of the initial spot, indicated successful advancement of the reaction. The synthesized compounds were purified by suitable workup procedures followed by recrystallization using appropriate solvents. Purity was further confirmed by conducting qualitative tests for functional groups to ensure the absence of starting materials or side products.

Methods:

General Synthesis Procedure:

Step I- Acetylation of Curcumin: Curcumin (3.00 g, 8.14 mmol, 1.0 equiv) was dissolved in anhydrous tetrahydrofuran (THF, 11.4 mL) in a dry round-bottom flask under continuous stirring. The reaction mixture was cooled to 0–5 °C using an ice bath, and acetyl chloride (1.28 mL, 18.0 mmol, 2.2 equiv) was added dropwise to the stirred solution to control the exothermic nature of the reaction. After complete addition, the reaction mixture was allowed to warm to room temperature and subsequently heated under reflux at 65–70 °C for 2–3 hours. Alternatively, for microwave-assisted synthesis, the reaction mixture was transferred to a microwave-compatible sealed vessel and irradiated at 420 W for 2–4 minutes, ensuring that the internal temperature did not exceed 80 °C; this method was used as a replacement for conventional reflux and not in combination with it.

The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel G-coated plates, with a mobile phase consisting of dichloromethane and methanol (5:1 v/v), where disappearance of the starting material spot indicated completion of the reaction. Upon completion, the reaction mixture was cooled to room temperature and carefully quenched by the slow addition of ice-cold distilled water (20 mL) under stirring. The mixture was then transferred to a separatory funnel and extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with saturated sodium bicarbonate solution to neutralize excess acetyl chloride, followed by brine solution (10 mL), then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was obtained and further purified, if necessary, by recrystallization using ethanol to yield the acetylated derivative of curcumin.

Step II- Biginelli Cyclocondensation Reaction [9]: Curcumin (0.736 g, 2.0 mmol, 1.0 equiv), the appropriate substituted aromatic aldehyde (2.0

mmol, 1.0 equiv; compounds 1A–8A), and urea or thiourea (3.0 mmol, 1.5 equiv; urea for selected derivatives and thiourea where thione analogues were intended) were introduced into a 50 mL round-bottom flask. The reaction was carried out using ethanol (2 mL) as the solvent, and phosphomolybdic acid ($H_3PMo_{12}O_{40}$, 5 mol%, 0.10 mmol) was added as the catalyst. The reaction mixture was stirred to ensure homogeneity and then subjected to either conventional reflux or microwave irradiation (not simultaneously). For the conventional method, the mixture was heated under reflux at 75–80 °C for 2–4 hours. For the microwave-assisted method, the reaction was transferred to a sealed microwave-compatible vessel and irradiated at 420 W for 150–210 seconds, maintaining the internal temperature in the range of 100–110 °C. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel G plates and a mobile phase of dichloromethane: methanol (5:1 v/v). Upon completion, the reaction mixture was cooled to room temperature, and approximately two-thirds of the solvent was removed under reduced pressure. The concentrated mixture was then poured into crushed ice to induce precipitation of the product. The resulting solid was collected by filtration, washed thoroughly with hot water to remove residual catalyst and impurities, and dried. Further purification was carried out using diethyl ether to obtain the final curcumin-based derivatives. The general reaction scheme is presented in **Fig. 1**, the smiles formula for synthesized compounds given in **Table 1** and the corresponding physical data are summarized in **Table 2.**

Spectral Characterization: Using a variety of analytical techniques, the synthesised compounds' structures were confirmed. The JASCO FT-IR V-460 Plus equipment was used to record infrared spectra of materials that were produced as potassium bromide pellets. The absorption bands were reported in units of wave numbers (cm^{-1}). Measurements of proton nuclear magnetic resonance (^1H-NMR) were performed using a VARIAN MERCURY YH spectrometer running at 300 MHz. Chemical shifts, measured in parts per million (ppm), were calibrated using tetramethylsilane (TMS) as the internal standard and deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. To obtain mass spectrometry data, a Shimadzu LC-MS device was utilised. The Central Instrument Facility in the Chemistry Department of Savitribai Phule Pune University served as the site for all experimental analyses.

Antibacterial Activity:

Minimum Inhibitory Concentration (MIC) Determination: The antibacterial activity of the synthesized curcumin derivatives was evaluated by determining the minimum inhibitory concentration (MIC) using the standard microbroth dilution method in accordance with established guidelines. The test compounds were initially dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with sterile Mueller–Hinton broth (MHB) to obtain the desired working concentrations. The final concentration of DMSO in all wells was maintained below 1% (v/v) to eliminate any potential solvent-induced effects on bacterial growth.

A two-fold serial dilution was performed to generate a concentration range of 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL. The assay was conducted in sterile 96-well microtiter plates, with each well containing a final volume of 200 µL, comprising equal volumes of compound solution and bacterial inoculum. Standard bacterial strains, including *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), were used for the study. The bacterial inoculum was prepared from freshly cultured colonies and adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL), followed by dilution to achieve a final inoculum density of ~5×10^5 CFU/mL in each well.

The microtiter plates were incubated at 37 °C for 18–24 hours under aerobic conditions. Following incubation, bacterial growth was assessed visually based on turbidity, and where necessary, further confirmed using a resazurin-based colorimetric indicator. The MIC was defined as the lowest concentration of the test compound that completely inhibited visible bacterial growth. Appropriate controls were included in each experiment: (i) sterility control (broth only), (ii) growth control (broth with inoculum but without test compound), (iii) solvent control (DMSO at the highest concentration used), and (iv) positive control using a standard antibiotic (e.g., ampicillin) tested over

an equivalent concentration range under identical conditions. All experiments were performed in triplicate and repeated independently at least twice to ensure reproducibility. Where applicable, results are expressed as mean ± standard deviation (SD), and consistency across independent experiments confirmed the reliability of the observed antibacterial activity.

TABLE 1: SMILES FORMULA FOR DESIGNED COMPOUNDS

Sr. no.	Smiles formula for compounds
1A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=C(C(C)=O)C=CC=C3)=O)=O)=CC=C1O
2A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=CC=CC=C3)=S)=O)=CC=C1O
3A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=C(C(C)=O)C=CC=C3)=O)=O)=CC=C1O
4A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=C(C(C)=O)C=CC=C3)=S)=O)=CC=C1O
5A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=CC(OC)=C(O)C=C3)=O)=O)=CC=C1O
6A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)N([H])C(N([H])C2C3=CC(OC)=C(O)C=C3)=S)=O)=CC=C1O
7A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)NC(NC2/C=C/C3=CC=CC=C3)=O)=O)=CC=C1O
8A	COC1=CC(/C=C/C(C2=C(/C=C/C4=CC=C(OC)C(OC(C)=O)=C4)NC(NC2/C=C/C3=CC=CC=C3)=S)=O)=CC=C1O

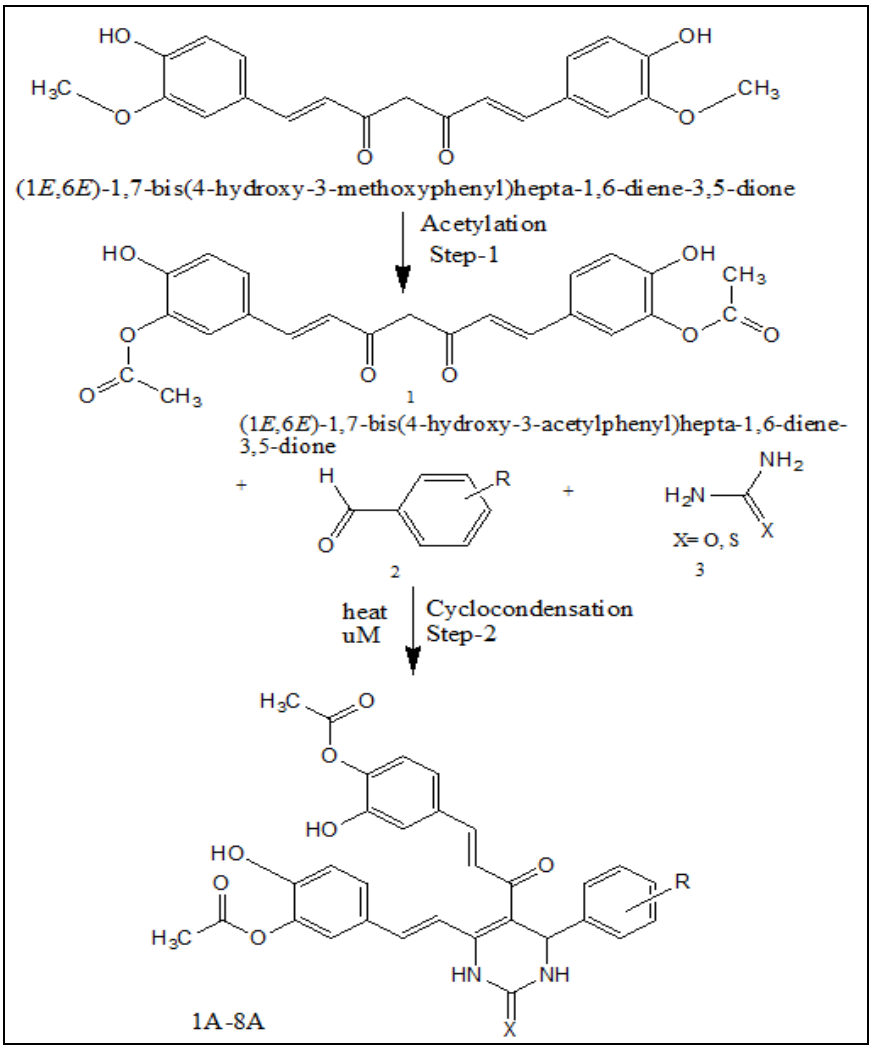

FIG. 1: SYNTHESIS OF 3,4 DIHYDROPYRIMIDINONES/THIONES OF ACETYLATED CURCUMIN 1A-8A UNDER MICROWAVE IRRADIATION

RESULT:

Ferric Chloride test & FTIR for Phenolic -OH group (Step I): This qualitative test relies on the reaction between phenolic compounds and neutral ferric chloride (Fe^{3+}) solution, leading to the formation of colored ferric-phenolate complexes. The color produced varies depending on the structure, phenol and the position of (-OH). Instandard curcumin, which contains monophenolic groups, a violet or blue coloration is typically observed. However, when this test was applied to the synthesized curcumin derivatives, no visible color change occurred, indicating potential modification or masking of the phenolic –OH groups. Test result were shown in **Fig. 2.**

FTIR image given for there is absene of phenolic OH stretch at 3300-3550 cm^{-1} indicating hydrogen bonding and presence of ester strong peak at 1722.38 cm^{-1} the graph shown in **Fig. 3.**

FIG. 2: FERRIC CHLORIDE TEST

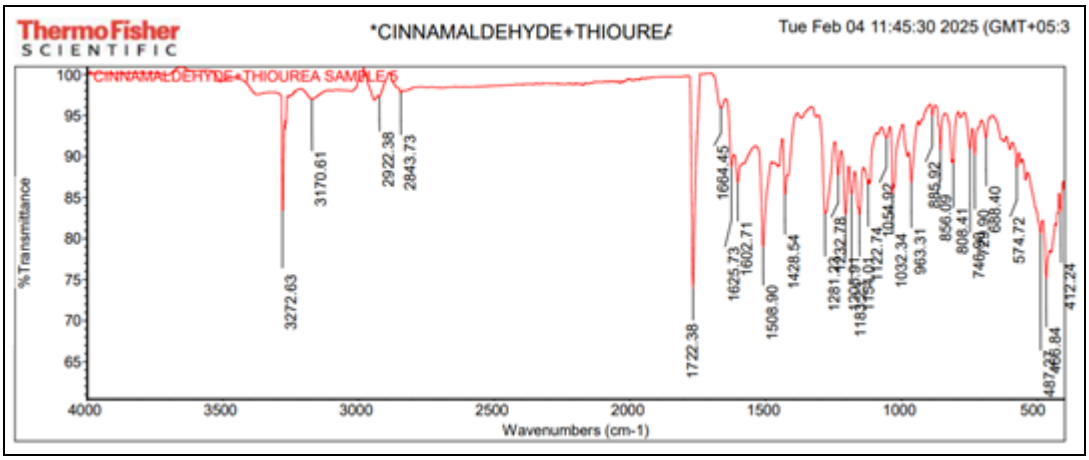

FIG. 3: FTIR FOR PHENOLIC OH GROUP

Thin Layer Chromatography (TLC) Analysis: TLC was used to monitor reaction progress and assess purity of the synthesized curcumin analogues. Analyses were performed on pre-coated silica gel 60 F_{254} aluminum plates (0.25 mm thickness) using dichloromethane:methanol (5:1, v/v) as the mobile phase. The developing chamber was pre-saturated for 20 minutes, and plates were developed to a distance of 7–8 cm. Spots were visualized under UV light (254 and 366 nm) and, when required, by iodine vapor. Completion of the reaction was confirmed by disappearance of starting material spots and appearance of a new product spot. All compounds showed single spots, indicating good purity. Rf values were calculated and reported as mean ± SD (n = 3) with a precision of ±0.01.

TABLE 2: THE RETENTION FACTOR (RF) VALUES OF *CURCUMIN ANALOGUES*

Compound code	Mobile phase (5:1)	Rf (mean ± SD)	Colour and physical appearance
1A	CH_2Cl_2 and CH_3OH	0.66 ± 0.01	Dark brown crystal
2A	CH_2Cl_2 and CH_3OH	0.72 ± 0.01	Brown crystal
3A	CH_2Cl_2 and CH_3OH	0.68 ± 0.01	Dark brown crystal
4A	CH_2Cl_2 and CH_3OH	0.72 ± 0.01	Dark brown crystal
5A	CH_2Cl_2 and CH_3OH	0.76 ± 0.01	Brown crystal
6A	CH_2Cl_2 and CH_3OH	0.68 ± 0.01	Dark brown crystal

7A	CH_2Cl_2 and CH_3OH	0.72 ± 0.01	Dark green crystal
8A	CH_2Cl_2 and CH_3OH	0.66 ± 0.01	Green crystals

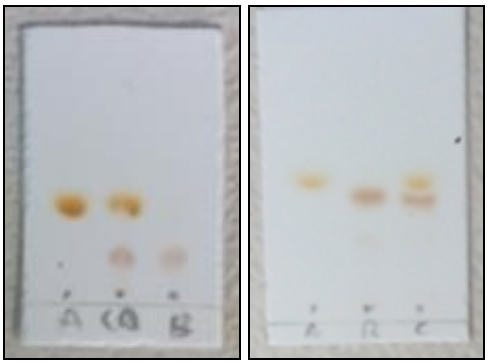

FIG. 4: THIN LAYER CHROMATOGRAPHY OF SYNTHESIZED COMPOUND STEP I & STEP II

Compound 1A: The IR spectrum (KBr) exhibited peaks at 3066 cm^{-1} (Ar C–H), 1735 and 1710 cm^{-1} (C=O), 1499 cm^{-1} (C=N), and 1491 cm^{-1} (Ar C=C). The ^1H NMR (DMSO-d_6) showed signals at δ 2.05–2.15 (6H, s, CH_3), 3.18 (1H, dd, Ha), 3.75 (3H, s, OCH_3), 3.83 (1H, dd, Hb), 3.92 (3H, s, OCH_3), 4.23 (1H, s), 5.15 (1H, dd, Hc), 6.58 (1H, d), 6.87 (1H, dd), and 7.06–7.78 (13H, m, Ar–H). LC–MS (ESI) showed m/z 584.2 $[M+H]^+$, consistent with the calculated mass.

Compound 2A: IR peaks were observed at 3169 cm^{-1} (Ar C–H), 3330 cm^{-1} (N–H), 1751 cm^{-1} (C=O, ester), 1626 cm^{-1} (Ar C=C), and 1602 cm^{-1} (C=O, urea). The ^1H NMR (DMSO-d_6) displayed δ 2.05–2.15 (6H, s, CH_3), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.23 (1H, s), 6.58 (1H, d), 6.87 (1H, dd), and 7.06–7.78 (13H, m, Ar–H). LC–MS gave m/z 599.2 $[M+H]^+$.

Compound 3A: The IR spectrum showed 3011 cm^{-1} (Ar C–H), 3334 cm^{-1} (N–H), 1727 cm^{-1} (C=O, ester), 1645 cm^{-1} (Ar C=C), and 1626 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) exhibited δ 2.05–2.25 (9H, s, CH_3), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.17 (1H, s), 6.57 (1H, d), 6.81–7.78 (12H, m, Ar–H), and 7.91 (1H, ddd). LC–MS showed m/z 625.2 $[M+H]^+$.

Compound 4A: IR bands appeared at 2922 cm^{-1} (Ar C–H), 3272 cm^{-1} (N–H), 1722 cm^{-1} (C=O, ester), 1664 cm^{-1} (C=C, cinnamic), and 1625 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) signals included δ 2.05–2.25 (9H, s, CH_3), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.17 (1H, s), 6.57 (1H, d), 6.81–7.78 (12H, m, Ar–H), and 7.91 (1H, ddd). LC–MS gave m/z 629.2 $[M+H]^+$.

Compound 5A: IR showed peaks at 3013 cm^{-1} (Ar C–H), 3329 cm^{-1} (N–H), 1715 cm^{-1} (C=O, ester), 1626 cm^{-1} (Ar C=C), and 1618 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) had δ 2.05–2.15 (6H, s, CH_3), 2.10 (3H, s), 3.70–3.92 (6H, s, OCH_3), 4.88 (1H, s), 6.52–6.72 (3H, m), 6.81–6.94 (2H, m), and 7.08–7.47 (5H, m, Ar–H). LC–MS: m/z 629.2 $[M+H]^+$.

Compound 6A: IR peaks at 3011 cm^{-1} (Ar C–H), 3334 cm^{-1} (N–H), 1727 cm^{-1} (C=O, ester), 1626 cm^{-1} (Ar C=C), 1618 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) δ 2.05–2.15 (6H, s, CH_3), 2.10 (3H, s, CH_3), 3.70–3.92 (6H, s, OCH_3), 4.88 (1H, s), 6.52–6.72 (3H, m), 6.81–6.94 (2H, m), 7.08–7.78 (8H, m, Ar–H). LC–MS: m/z 645.2 $[M+H]^+$.

Compound 7A: IR bands at 3012 cm^{-1} (Ar C–H), 3321 cm^{-1} (O–H), 1710 cm^{-1} (C=O, ester), 1625 cm^{-1} (Ar C=C), 1662 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) δ 2.05–2.15 (6H, s, CH_3), 2.10 (3H, s), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.73 (1H, d), 6.39–6.65 (3H, m), 6.87 (1H, dd), 7.06–7.78 (13H, m, Ar–H). LC–MS: m/z 607.1 $[M+H]^+$.

Compound 8A: IR peaks at 3011 cm^{-1} (Ar C–H), 3306 cm^{-1} (O–H), 1713 cm^{-1} (C=O, ester), 1626 cm^{-1} (Ar C=C), 1618 cm^{-1} (C=O, urea). ^1H NMR (DMSO-d_6) δ 2.05–2.15 (6H, s, CH_3), 2.10 (3H, s), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.73 (1H, d), 6.39–6.65 (3H, m), 6.87 (1H, dd), 7.06–7.78 (13H, m, Ar–H). LC–MS: m/z 607.1 $[M+H]^+$.

Biological Evaluation (Antibacterial Activity): Among the compounds tested against *Escherichia coli*, compound 4A showed the strongest antibacterial effect, exhibiting the lowest minimum inhibitory concentration (MIC) of 9 µg/mL. This was closely followed by compounds 2A and 8A, with MIC values of 10 µg/mL and 11 µg/mL, respectively. Notably, these derivatives outperformed the reference antibiotic Ampicillin, which had an MIC of 16 µg/mL. Additionally, curcumin (14 µg/mL), as well as compounds 5A (12 µg/mL) and 6A (15 µg/mL), showed considerable antibacterial activity. The least potent among the series were compounds 3A and 7A, with MIC values of 24 µg/mL and 19 µg/mL, respectively.

FIG. 5: BROTH DILUTION METHOD ZONE OF INHIBITION COMPARING WITH STD. AMPICILLIN

TABLE 3: MINIMUM INHIBITORY CONCENTRATION FOR *E. COLI* BACTERIA OF CURCUMIN ANALOGUES

Sr. no.	Name of Compound	MIC (µg/mL) (mean ± SD, n = 3, 2 independent experiments)
1	Curcumin	14 ± 0.58
2	Standard Ampicillin	16 ± 0.82
3	1A	18 ± 1.0
4	2A	10 ± 0.58
5	3A	24 ± 1.15
6	4A	9 ± 0.58
7	5A	12 ± 0.58
8	6A	15 ± 0.58
9	7A	19 ± 0.82
10	8A	11 ± 0.58

CONCLUSION: A series of eight innovative curcumin derivatives (1A to 8A) were synthesized, and their molecular structures were confirmed by employing spectroscopic techniques such as IR, NMR, and mass spectrometry.

The antibacterial efficacy of these compounds was evaluated against *Escherichia coli*, with the Minimum Inhibitory Concentration results revealing that several derivatives exhibited greater activity than both native curcumin and the reference antibiotic Ampicillin. Notably, compounds 4A, 2A, and 8A showed MIC values of 9, 10, 11 micrograms per milliliter, respectively, indicating improved antibacterial potency. These findings suggest that chemical modifications of curcumin can enhance its antibacterial properties, with compound 4A emerging as a particularly promising candidate for further antibacterial drug development.

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CONFLICTS OF INTEREST: Nil

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