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Synthesis, Characterization, and Biological Evaluation of Novel N- (Substituted Aryl) Acryloyl Theophylline Derivatives as Potential Antimicrobial, Anti-Inflammatory, and Anti-Asthmatic Agents

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ABSTRACT

The acryloyl group has significant medicinal importance due to its chemical reactivity and ability to form covalent bonds with other molecules, which makes it useful in various pharmaceutical, therapeutic, and biomedical applications. N-(Substituted aryl) acryloyl theophylline derivatives [3(a–e)] were synthesized through a Claisen–Schmidt condensation reaction involving various aromatic aldehydes and acetopurinone derivatives in ethanol under alkaline (40% NaOH) or acidic (hydrochloric acid-catalyzed) conditions. The resulting products were isolated and purified by recrystallization to obtain analytically pure compounds. Structural characterization of the synthesized derivatives was carried out using Fourier Transform Infrared (FT-IR) spectroscopy and Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy. The biological potential of the newly synthesized compounds was subsequently evaluated through antimicrobial screening using the Kirby–Bauer disc diffusion assay, anti-inflammatory activity using the carrageenan-induced paw edema model, and bronchodilator efficacy employing the histamine-induced bronchospasm method.

Keywords: *N- (substituted aryl) acryloyl theophylline derivatives, Kirby - Bauer Disc Diffusion method, anti-inflammatory, Bronchodilator activity.*

1. Introduction

N- (Substituted Aryl) Acryloyl Theophylline Derivatives are a class of compounds that involve the combination of theophylline, a known methylxanthine derivative, with acryloyl groups and substituted aryl groups. These derivatives are of interest in medicinal chemistry due to their potential bioactivity, which could include stimulating effects on the central nervous system, bronchodilation (opening of airways), or other therapeutic activities. When attached to theophylline, the acryloyl group can modify the molecule's solubility, bioavailability, and pharmacokinetics, potentially improving its therapeutic efficacy. The aryl group refers to an aromatic ring structure, such as a benzene ring, attached to the molecule. The presence of substituted aryl groups means that the aromatic ring has one or more functional groups (like methoxy, hydroxyl, chloro, etc.) attached to it. These substitutions can influence the molecule's lipophilicity, polarizability, and binding affinity for biological targets. The study of N-(substituted aryl) acryloyl theophylline derivatives is an exciting area in medicinal chemistry, aiming to leverage the therapeutic benefits of theophylline while modifying its structure to improve drug delivery, pharmacodynamics, and targeting. The combination of theophylline, acryloyl, and aryl substitutions offers the potential for more efficient, controlled, and targeted therapies, particularly for respiratory conditions or related disorders [1-12] (anti-microbial, anti-inflammatory effects).

2. Materials and Methods

All reagents and solvents employed in this study were of analytical grade and were obtained from commercial suppliers. The majority of the chemicals were procured from Aldrich Chemical Corporation and used as received without further purification. Melting points of the synthesized compounds were determined using the open capillary tube method and are reported without correction. The progress of the reactions and the purity of the synthesized compounds were monitored by thin-layer chromatography (TLC) using aluminum-backed silica gel plates (0.25 mm thickness). Chromatographic spots were detected under ultraviolet (UV) light and further visualized using iodine vapor. Fourier Transform Infrared (FT-IR) spectra were recorded on a Shimadzu FT-IR spectrophotometer using KBr pellet techniques, and absorption frequencies are reported in cm^{-1}. Proton Nuclear Magnetic Resonance (^1H NMR) spectra were acquired on a Bruker spectrometer operating at 500 MHz using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the internal reference standard.

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Chemical shifts are expressed as δ values in parts per million (ppm). Signal multiplicities are designated as singlet (s), doublet (d), and multiplet (m), while coupling constants (J) are reported in Hertz (Hz). The elemental composition and molecular properties of the synthesized compounds were analyzed using ChemSketch software to support structural characterization.

2.1. Synthesis and Characterization

of several bioactive heterocyclic compounds. Many common medicines available for different diseases are found to contain 1,2,4-triazole as a heterocyclic moiety. The examples include Ribavirin, which is an antiviral drug, Rizatriptan is used to treat migraines, Estazolam and Alprazolam are anxiolytic, Letrozole and Anastrozole are anticancer drugs. Triazole derivatives found in drugs like Itraconazole, Fluconazole, and Posaconazole are useful for the treatment of fungal infections, whereas Ru conamide is a well-known anticonvulsant [8-19]. Triazole derivatives also found to possess moderate to good antibacterial and antifungal activities [20]. Many methods are reported for the preparation of bioactivetriazole derivatives. One of them is Biginelli reaction, which involves the condensation of 1,2,4-triazole-5-amine and β-keto ester with different aldehydes. Looking to the pharmacological importance, we have synthesized a new series of compounds containing triazole and dihydropyrimidine moieties in one framework using the reported method [21, 22].

Materials and Methods

General: All the chemicals required are obtained from Spectrochem, Finar, and Sigma Aldrich. Merck Kieselgel 60 F254 plates were recorded in DMSO-d6 solution in 5 mm tubes at room temperature, on a BRUKER 400 MHz FT-NMR, with TMS as internal standard. IR Spectra were recorded on a SHIMADZU FT-IR 8400 using potassium bromide pellets. Mass spectra were recorded on SHIMADZU QP-2010. The antimicrobial activity was carried out using the broth dilution method to determine the minimum inhibitory concentration (MIC).

2.1.1. Synthesis of sodium salt of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (1)

To theophylline (0.04mol or 7.3gm) in a stoppered conical flask, was added sodium metal (0.04 mol, 0.88 gm), little by little, till effervescence ceased. Solid separation on cooling was used as such for the next step.

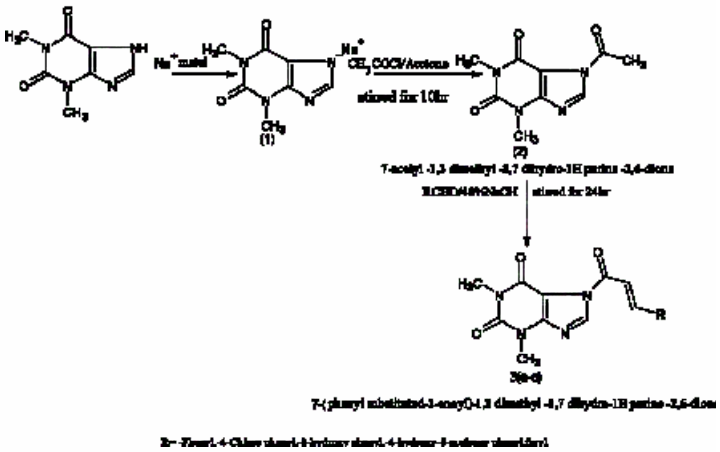
2.1.2. Synthesis of 7-acetyl-1, 3dimethyl-3, 7 dihydro-1H purine-2, 6-dione (2)

The sodium salt of theophylline (1) (0.03mol or 5.2 gm) was suspended in acetone and acetyl chloride (0.03mol, 2.5ml) while stirring and cooling. It was stirred for 1 hr. and K_2CO_3 (0.2mol or 28 g) was added as a base. It was further stirred for another 10 hours. The progress and completion of the reaction were confirmed by TLC (Mobile Phase 7.5:2:0.5 Toluene: Methanol: Ammonia). A solid was obtained after the distillation of acetone and recrystallized in ethanol.

2.1.3. Synthesis of 1,3-dimethyl-7-(-3-aryl substituted prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione 3(a-e)

N-Acetyl theophylline (2) (0.01 mol, 2.23 g) was dissolved in 20 mL of ethanol and reacted with the appropriate aromatic aldehyde (0.01 mol).

Subsequently, 40% aqueous sodium hydroxide solution was added dropwise, and the reaction mixture was stirred continuously at room temperature for 24 h. The progress of the reaction was monitored by thin-layer chromatography (TLC) using a chloroform (9:1, v/v) solvent system as the mobile phase. Upon completion of the reaction, the mixture was poured onto crushed ice and acidified with 10% hydrochloric acid to facilitate product precipitation. The resulting solid was collected by filtration, washed with cold water to remove residual impurities, and dried. The crude products were purified by recrystallization from aqueous ethanol to afford the desired N-(substituted aryl) acryloyl theophylline derivatives (3a–3e) in good yield. The synthetic route employed for the preparation of the target compounds is presented in Scheme 1.



2.1.4. Physical Characterization

Table: 1. Physical characterization of synthesized 1,3-dimethyl-7-(-3-aryl substituted prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione derivatives 3(a-e):

S.No.	Compounds (R)	%Yield W/W	Melting point (°C)	Rf value	Molecular Formula	Molecular Weight	Composition %				
							C	H	N	O	Cl
3a	phenyl	72.68	265-268	0.59	$C_{16}H_{14}N_4O_3$	310.30	61.93	4.55	18.06	15.47	
3b	4-chloro phenyl	69.48	252-256	0.67	$C_{16}H_{13}ClN_4O_3$	344.75	55.74	3.80	16.25	13.92	10.28
3c	2-hydroxy phenyl	65.43	246-248	0.76	$C_{16}H_{14}N_4O_4$	326.30	58.89	4.32	17.17	19.61	
3d	4-hydroxy 3-methoxy phenyl	72.78	269-271	0.70	$C_{17}H_{16}N_4O_5$	356.33	57.30	4.53	15.72	22.45	
3e	2-furyl	79.80	210-213	0.81	$C_{14}H_{12}N_4O_4$	300.26	56.00	4.03	18.66	21.31	

Mobile phase - Chloroform: Methanol (9:1)

2.1.5. Spectral Characterization

Table: 2. IR & ^{1}H NMR spectrum data of synthesized 1,3-dimethyl-7-(-3-aryl substituted prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione derivatives 3(a-e):

S. No.	Compounds (R)	IR frequency region(cm-1)	^{1}H NMR (δ ppm)	Mass (m/z)
3a	phenyl	3005.2 (C-H stretching in aromatic), 2808.15 (N-CH_3 stretching), 1683.98 (C=C stretching), 1660.55 (C=N Stretching), 1637.97 (C=O Stretching), 1531.53 (C=C stretching in aromatic), 1315.86 (C-N stretching).	2.77(3H, s), 2.97(3H, s), 6.84-7.24(5H, m) 7.53(1H, d), 7.80(1H, d).	309
3b	4-chloro phenyl	3088.41 (C-H stretching in aromatic), 2811.55 (N-CH_3 stretching), 1716.98 (C=C stretching), 1681.55) (C=N Stretching), 1637.97 (C=O Stretching), 1531.53 (C=C stretching in aromatic), 1315.86 (C-N stretching), 744 (C-Cl stretching).	2.77(3H, s), 2.97 (3H, s), 6.84-7.24(4H,m) 7.50(1H, d) 7.74 (1H, d)	343
3c	2-hydroxy phenyl	3460.41 (OH stretching), 3060.41 (C-H stretching in aromatic), 2815.55 (N-CH_3 stretching), 1771.98 (C=C stretching), 1666.55 (C=N Stretching), 1627.97 (C=O Stretching), 1531.53 (C=C stretching in aromatic), 1332.86 (C-N stretching).	2.77(3H, s), 2.97 (3H, s), 6.84-7.24(4H,m) 7.32(1H,d) 7.78(1H,d), 9.95(1H, s).	326
3d	4-hydroxy 3-methoxy phenyl	3511(OH stretching), 3086.21 (C-H stretching in aromatic), 2815.55 (N-CH3 stretching), 1681.98 (C=C stretching), 1666.55 (C=N Stretching), 1627.97 (C=O Stretching), 1531.53 (C=C stretching in aromatic), 1332.86 (C-N stretching), 1172.76 (C-O-C stretching).	2.77(3H,s), 2.97(3H,s), 3.87(3H,s), 6.5-7.2(3H,m) 7.41(1H, d), 7.78(1H,d), 9.95(1H, s).	356
3e	2-furyl	3086.21 (C-H stretching in aromatic), 2815.15 (N-CH_3stretching), 1683.91 (C=Cstretching), 1660.77(C=NStretching), 1627.97(C=OStretching), 1531.53 (C=Cstretching in aromatic), 1330.93 (C-N stretching), 1170.83 (C-O-C Stretching).	2.77(3H,s), 2.97 (3H, s), 3.15-3.25(3H,m) 7.54 (1H,d), 7.79 (1H, d)	300

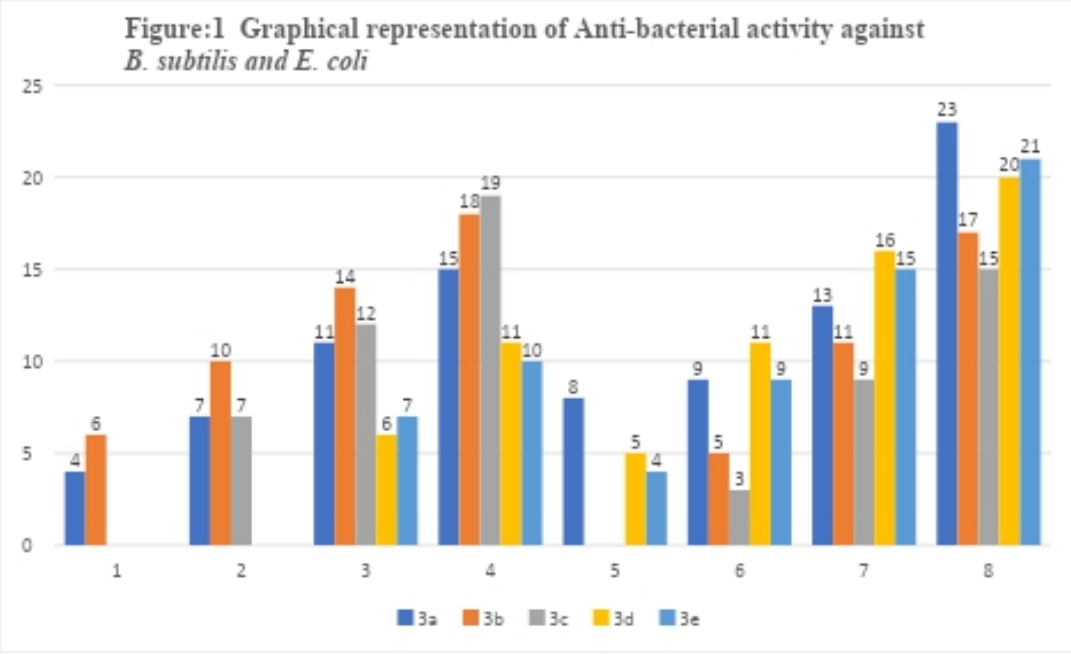
3. Evaluation of pharmacological activities

3.1 *In Vitro* Antimicrobial Screening

The antibacterial activity of the synthesized compounds was evaluated using the Kirby–Bauer disc diffusion method. The test organisms included one Gram-positive bacterium, *Bacillus subtilis*, and one Gram-negative bacterium, *Escherichia coli*. Nutrient agar medium was prepared by dissolving beef extract (1.0 g), peptone (1.0 g), and sodium chloride (0.5 g) in 100 mL of double-distilled water. The medium was sterilized by autoclaving and subsequently poured into sterile Petri dishes. After solidification, the agar plates were inoculated with freshly prepared overnight bacterial cultures using a sterile spreader to ensure uniform distribution of the microorganisms. Sterile Whatman filter paper discs (5 mm diameter) were impregnated with different concentrations (1.0, 2.5, 5.0, and 10.0 mg) of the synthesized compounds and carefully placed on the surface of the inoculated agar plates. Ciprofloxacin (20 µg/disc) was used as the reference antibacterial drug. The plates were incubated at 37°C for 24 h under aseptic conditions. Following incubation, antibacterial activity was assessed by measuring the diameter of the clear zone of inhibition surrounding each disc. The extent of microbial growth inhibition was expressed in millimeters (mm), and larger inhibition zones were considered indicative of greater antibacterial efficacy of the tested compounds.

Table 3: Zone of Inhibition (mm) for Different Compounds Against B. subtilis and E. coli at Varying Concentrations

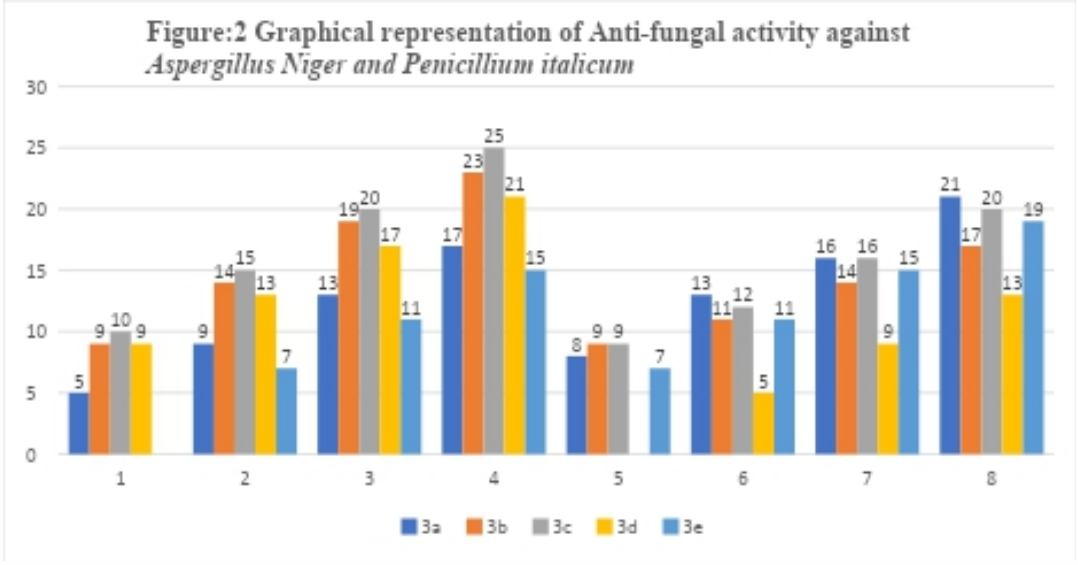
S. No.	Compound	Concentration of the zone of inhibition in mm							
		B. subtilis (Gram +ve)				*E. coli* (Gram – ve)			
		1mg	2.5mg	5mg	10mg	1mg	2.5mg	5mg	10mg
3a	phenyl	4	7	11	15	8	9	13	23
3b	4-chloro phenyl	6	10	14	18	-	5	11	17
3c	2-hydroxy phenyl	-	7	12	19	-	3	9	15
3d	4-hydroxy 3-methoxy phenyl	-	-	6	11	5	11	16	20
3e	2-furyl	-	-	7	10	4	9	15	21
	Control	-				-			



For *B. subtilis* (Gram-positive), the zone of inhibition increased with the concentration of the compounds, with phenyl (3a) showing the largest zone of inhibition at 10 mg (15 mm). For *E. coli* (Gram-negative), phenyl (3a) and 4-chloro phenyl (3b) also exhibited substantial activity, with phenyl reaching the largest zone of inhibition (23 mm) at 10 mg. Some compounds, like 2-hydroxy phenyl (3c) and 2-furyl (3e), showed minimal to no activity at lower concentrations but exhibited larger inhibition zones as the concentration increased.

Table 4: Zone of Inhibition (mm) for Different Compounds Against Aspergillus niger and Penicillium italicum at varying Concentrations

S.No.	Compound	Concentration of the zone of inhibition in mm							
		Aspergillus Niger				*Penicillium italicum*			
		1mg	2.5mg	5mg	10mg	1mg	2.5mg	5mg	10mg
3a	phenyl	5	9	13	17	8	13	16	21
3b	4-chloro phenyl	9	14	19	23	9	11	14	17
3c	2-hydroxy phenyl	10	15	20	25	9	12	16	20
3d	4-hydroxy 3-methoxy phenyl	9	13	17	21	-	5	9	13
3e	2-furyl	-	**7**	11	15	7	11	15	19
	Control	-				-			



For *Aspergillus niger,* all compounds showed a dose-dependent increase in the zone of inhibition as concentration increased. The compound 2-hydroxy phenyl (3c) exhibited the largest inhibition zone (25 mm) at 10 mg. For *Penicillium italicum*, the compounds also demonstrated increased inhibition zones with higher concentrations. Again, 2-hydroxy phenyl (3c) showed the greatest activity at 10 mg, with a zone of inhibition of 20 mm. Compounds like phenyl (3a) and 4-chloro phenyl (3b) were also effective but did not reach the level of 2-hydroxy phenyl (3c), especially at higher concentrations. The control did not show any zone of inhibition, confirming the activity is due to the compounds being tested. Phenyl (3a), 4-chloro phenyl(3b), 2-hydroxyphenyl (3c), and substituted at C7 showed significant activity against *Aspergillus Niger and Penicillium italicum* fungi except for 4-hydroxy 3-methoxy phenyl (3d) and furan-2-yl(3e). 2- hydroxyphenyl (3c), 4-chloro phenyl(3b) and 4-hydroxy 3-methoxy phenyl (3d), substituted at C7 displayed remarkable activity against *Aspergillus Niger)* and phenyl (3a), 2-hydroxyphenyl (3c), and 4-hydroxy 3-methoxy phenyl (3d) substituted at C7 displayed remarkable activity against *Penicillium italicum*.

3.2 Anti-Inflammatory Activity

The anti-inflammatory activity of the synthesized compounds was evaluated using the carrageenan-induced hind paw edema model in rats, a widely accepted method for assessing acute inflammation. Acute inflammation was induced by subplantar injection of 0.1 mL of a 1% carrageenan suspension prepared in normal saline using 2% gum acacia as the suspending agent. The test compounds were administered according to the experimental design before carrageenan injection. Indomethacin sodium (50 mg kg^{-1}, p.o.), suspended in 0.5% carboxymethyl cellulose (CMC), was used as the reference anti-inflammatory drug. One hour after treatment administration, carrageenan was injected into the right hind paw of each animal to induce edema. Paw volume was measured immediately before carrageenan administration (0 h) and subsequently at 3 and 5 h post-injection using Vernier calipers. The anti-inflammatory effect of the synthesized compounds was determined by comparing the paw edema in treated groups with that of the control group.

Drugs and chemicals

Indomethacin sodium (Merck), Carrageenan (Sigma Aldrich Chemical Co.), 0.1% solution of SCMC, 2% gum acacia, normal saline, and 0.5% CMC were used in this study.

Materials and Methods

The anti-inflammatory was treated with the test compounds 3(a-e) at the doses of 50 mg/kg, p.o., and evaluated by Carrageenan acetic in rats injected subplantar administration with a dose of 1% (0.1ml/ body weight). Indomethacin sodium (25 mg/kg) p.o. was used as a standard. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's test. Procedure. The male rats (120-190g) were divided into four groups (n = 6) and fasted overnight before the experiment with free access to water. Group I: control group animals received 0.1% solution of SCMC only. Group II: standard group animals received Indomethacin sodium (IND 50 mg/kg p.o) dissolved in 0.5% CMC. Group III: The synthesized compounds 3(a-e) were suspended in 0.1% carboxymethylcellulose (SCMC) at doses of 50 mg/kg orally. After 1 hr, acute inflammation was produced by the subplantar administration of 0.1 ml of 1% suspension of Carrageenan (in SCMC w/v) into the plantar tissue of the right hind paw of the rats. After Carrageenan injection, the paw thickness was measured at 0 h, 4 h (0h -4h) by using Vernier calipers. The animals were pretreated with the drug 1 hour before the administration of Carrageenan. Anti-inflammatory activity was expressed as a percentage of inhibition of the inflammation when compared with the vehicle control group. The results obtained are tabulated in the table. The percentage inhibition of the inflammation was determined by the formula: % I = 1–(dt/dc) × 100, where "dt" is the difference in paw volume in the drug-treated group and "dc" is the difference in paw volume in the control group. Furthermore, "I" stands for inhibition of inflammation.

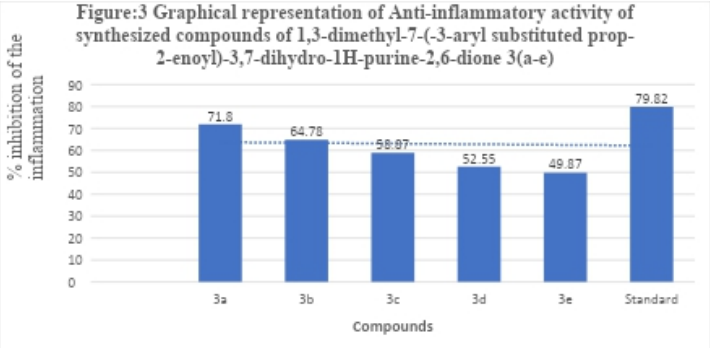
$$\%I = 1-(dt/dc) \times 100$$

where:

- (dt) = Mean increase in paw thickness in the treated group
- (dc) = Mean increase in paw thickness in the control group

Table 5: Anti-inflammatory activity of synthesized compounds of 1,3-dimethyl-7-(-3-aryl substituted prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione 3(a-e).

Animal group	R	% inflammation at the time of intervals		% inhibition of the inflammation
		0 hr	4 hr	
3a	phenyl	3.57±0.128	3.76±0.14**	71.80
3b	4-chloro phenyl	3.38±0.012	3.77±0.016*	64.78
3c	2-hydroxy phenyl	3.43±0.003	3.89±0.02	58.87
3d	4-hydroxy 3-methoxyphenyl	3.35±0.22	3.88±0.12	52.55
3e	2-furyl	3.31±0.12	3.87±0.23	49.87
Control	Vehicle	3.61±0.016	4.72±0.025	-
Standard	Indomethacin sodium	3.46±0.088	3.68±0.11	79.82



The % inflammation at 0 hours shows the initial inflammation level in the animal groups, with the vehicle control group showing the highest value (3.61±0.016). After 4 hours, the inflammation decreased in all experimental groups, with varying degrees of effectiveness. The % inhibition of inflammation was calculated based on the reduction in inflammation compared to the control group, and phenyl (3a) exhibited the highest inhibition (71.80%), followed by 4-chloro phenyl (3b) (64.78%).

3.3 Bronchodilator Activity

Experimental Animals

The bronchodilator activity of the synthesized compound was evaluated using a histamine-induced bronchospasm model in guinea pigs. Healthy guinea pigs of either sex weighing 350–500 g were procured and maintained under standard laboratory conditions at a temperature of 22 ± 2°C, relative humidity of 60 ± 5%, and a 12 h light/dark cycle. Animals were provided with a standard pellet diet and water ad libitum. Before the experiment, the animals were fasted for 24 h with free access to water.

The animals were randomly divided into three groups (n = 6):

- **Group I (Control):** Received normal saline or vehicle (1% sodium carboxymethyl cellulose, SCMC).
- **Group II (Standard):** Received pheniramine maleate (1 mg kg^{-1}, intraperitoneally).
- **Group III (Test):** Received synthesized compound 3(a) at a dose of 10 mg kg^{-1} orally, suspended in 1% SCMC.

Histamine-Induced Bronchospasm Assay

Bronchodilator activity was assessed using histamine aerosol-induced bronchospasm. Guinea pigs were exposed to a 0.2% histamine dihydrochloride aerosol prepared in normal saline using a nebulizer operating at a pressure of 300 mmHg. The aerosol was administered in a transparent Plexiglas chamber (24 × 14 × 24 cm). Exposure to histamine aerosol induced progressive respiratory distress characterized by dyspnea, bronchospasm, convulsions, and, ultimately, asphyxia. The time interval between the initiation of histamine exposure and the onset of pre-convulsive dyspnea (PCD) was recorded and used as an indicator of bronchodilator activity. Three days before the experiment, animals were screened for their responsiveness to histamine aerosol. Only guinea pigs exhibiting pre-convulsive dyspnea within 3 min of exposure were selected for the study. During the experiment, animals reaching the pre-convulsive stage were immediately removed from the chamber and allowed to recover in fresh air. The standard drug and test compound were administered 30 min before histamine exposure. Animals that did not develop characteristic bronchospasm within 6 min of exposure were considered protected.

Calculation of Percentage Protection

Bronchoprotective activity was expressed as percentage protection against histamine-induced bronchospasm and was calculated using the following formula:

Percentage protection = [(T2-T1)/T2] ×100.

where:

- (T_1) = Pre-convulsive dyspnea time before treatment
- (T_2) = Pre-convulsive dyspnea time after treatment

An increase in pre-convulsive dyspnea time indicated bronchodilator and antihistaminic activity of the test compound.

Table 6: Effect of synthesized compounds of 1,3-dimethyl-7-(-3-phenyl prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione 3(a) on histamine-aerosol in guinea pigs

Treatment	Dose	Preconvulsion time (min)	Protection
Control	Saline	5.50±0.01	-
Pheniramine maleate	1 mg/kg i.p	20.23±0.01	72.81
synthesized compounds 3(a)	10 mg/kg orally	16.35 ±0.01	66.36

p-value < 0.01.

Presenting data from an experiment assessing the effects of synthesized compound 3(a) on histamine-induced convulsions in guinea pigs, comparing it to a control and pheniramine maleate, a known antihistamine. The p-value is <0.01, meaning the differences between the groups (control, pheniramine, and synthesized compound 3(a)) are statistically significant.

Results and Discussion

4. Results and Discussion

Five novel 7-chalcone-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione derivatives (3a–3e) bearing different aromatic substituents were successfully synthesized through a Claisen–Schmidt condensation reaction under alkaline conditions. The reaction proceeded smoothly, affording the target compounds in moderate to good yields ranging from 65% to 79%. The progress of the reactions was monitored by thin-layer chromatography (TLC), which confirmed the formation of the desired products. Following synthesis, the crude compounds were purified by recrystallization from aqueous ethanol to obtain analytically pure products suitable for characterization and biological evaluation.

The chemical structures of the synthesized derivatives were established using FT-IR, ^1H NMR, and mass spectrometric analyses. The spectral data were in good agreement with the proposed molecular structures. FT-IR spectroscopy revealed characteristic absorption bands corresponding to the major functional groups present in the synthesized compounds. Broad absorption peaks observed in the region of 3511–3460 cm^{-1} were attributed to O–H stretching vibrations in hydroxyl-substituted derivatives. Aromatic C–H stretching vibrations appeared between 3088 and 3005 cm^{-1}, while the bands observed around 2815–2808 cm^{-1} were assigned to N–CH_3 stretching vibrations of the dimethylxanthine moiety. Strong absorption bands in the range of 1681–1771 cm^{-1} confirmed the presence of carbonyl (C=O) groups and conjugated carbon-carbon double bonds associated with the chalcone framework. Additional bands observed between 1681–1624 cm^{-1} were attributed to C=N and conjugated carbonyl stretching vibrations. The presence of C–O–C and C–N functionalities was supported by absorption bands at approximately 1172 cm^{-1} and 1332–1315 cm^{-1}, respectively. Furthermore, compounds containing chloro-substituted aromatic rings exhibited characteristic C–Cl stretching vibrations around 744 cm^{-1}.

The ^1H NMR spectra further substantiated the assigned structures. All compounds displayed two distinct singlets at approximately δ 2.77 and δ 2.97 ppm, corresponding to the methyl protons attached to the nitrogen atoms of the purine nucleus. Signals arising from aromatic and olefinic protons appeared as multiplets within the δ 6.46–7.95 ppm range, consistent with the expected proton environments of the chalcone derivatives. The integration values matched the predicted number of protons for each structure, confirming the substitution patterns on the aromatic rings. In compound 3b, a downfield singlet observed at δ 9.95 ppm was assigned to a phenolic hydroxyl proton, indicating the presence of a hydroxyl-substituted aromatic ring. Mass spectrometric analysis further supported the molecular compositions of the synthesized compounds through the observation of molecular ion peaks corresponding to their calculated molecular weights. The spectroscopic data conclusively confirmed the successful synthesis of the target 7-chalcone-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione derivatives, providing a strong basis for their subsequent biological evaluation.

Antibacterial activity

The data clearly show a dose-dependent increase in the zone of inhibition for both *B. subtilis* and *E. coli.* Higher concentrations of the compounds generally resulted in larger inhibition zones, indicating an increase in antimicrobial activity with higher compound concentrations. This aligns with typical antimicrobial testing, where an increased concentration of an antimicrobial agent often results in greater effectiveness. Among the compounds tested, phenyl (3a) was the most effective against both *B. subtilis* and *E. coli*, showing the largest zones of inhibition, especially at 10 mg. The presence of the phenyl group may contribute to its antimicrobial activity, possibly due to its ability to interact with bacterial cell membranes or enzymes, disrupting their function. This is consistent with studies where phenyl derivatives have shown antimicrobial potential.4-chloro phenyl (3b) also exhibited strong antibacterial activity, especially against *B. subtilis*, with a significant increase in zone of inhibition at 10 mg. However, its activity against E. coli was lower compared to phenyl (3a). The addition of a chlorine atom could enhance the compound's ability to penetrate bacterial cells, but the activity was less pronounced in *E. coli*, possibly due to the differences in membrane structures between Gram-positive and Gram-negative bacteria. Compounds like 2-hydroxy phenyl (3c) and 2-furyl (3e) showed delayed responses in terms of effectiveness, with stronger activity only seen at 5 mg and 10 mg concentrations. The hydrophobic nature of the furan and hydroxy groups may explain their relatively delayed onset of activity, as they could require higher concentrations to reach an effective concentration in the bacterial environment. The Gram-positive bacteria *B. subtilis* appeared to be more susceptible to all tested compounds, showing clear zones of inhibition even at lower concentrations. This is likely due to the simpler cell wall structure of Gram-positive bacteria, which is more susceptible to disruption by antimicrobial compounds. On the other hand, *E. coli*, a Gram-negative bacterium, showed less susceptibility overall. The outer membrane of *E. coli* provides an additional barrier to the diffusion of antimicrobial agents, making Gram-negative bacteria more resistant to many types of antimicrobial compounds. This could explain why the compounds required higher concentrations to show significant inhibition against *E. coli.* The control did not show any zone of inhibition, confirming that the observed antibacterial activity is solely due to the tested compounds and not any external factors. This experiment demonstrates the antimicrobial potential of the tested phenyl derivatives, with the phenyl compound (3a) showing the most promise. However, further studies, including testing with different concentrations and more bacterial strains, are needed to confirm the broad-spectrum activity and explore the mechanism behind the antibacterial effects of these compounds.

Antifungal activity

The zone of inhibition increased with the concentration of the tested compounds for both *Aspergillus niger* and *Penicillium italicum***,** indicating a dose-dependent effect. This is consistent with typical antimicrobial or antifungal tests, where increasing the concentration of an active agent results in a larger area of inhibition. Higher concentrations of the compounds lead to greater interaction with fungal cell walls and metabolic pathways, thus enhancing antifungal activity. Among the tested compounds, 2-hydroxyphenyl (3c) was the most effective against both *Aspergillus niger* and *Penicillium italicum***,** with the largest inhibition zones observed at the highest concentrations (25 mm for *Aspergillus niger* and 20 mm for *Penicillium italicum).* The presence of a hydroxyl group in 2-hydroxyphenyl might contribute to its strong antifungal activity by affecting the fungal cell membrane or enzymatic processes, as phenolic compounds are known to have potent antimicrobial and antifungal properties**.** Phenyl (3a) and 4-chloro phenyl (3b) also showed significant antifungal activity, but their maximum inhibition zones were slightly smaller than that of 2-hydroxy phenyl (3c)**,** with the highest being 23 mm for *Aspergillus niger* at 10 mg for 4-chloro phenyl (3b)**.** The addition of a chlorine atom in 4-chloro phenyl may have enhanced its ability to interact with fungal cells, but the increased activity was still not as high as that observed with 2-hydroxy phenyl.4-hydroxy 3-methoxy phenyl (3d) demonstrated moderate activity against *Aspergillus niger* with a maximum zone of inhibition of 21 mm at 10 mg, but was less effective against *Penicillium italicum*, with only a slight increase in activity observed with increasing concentration.2-furyl (3e) showed moderate activity as well, especially against *Aspergillus niger*, where the zone of inhibition increased from 7 mm at 2.5 mg to 15 mm at 10 mg. It was somewhat less effective against *Penicillium italicum* but still demonstrated a clear antifungal effect. Both *Aspergillus niger* and *Penicillium italicum* showed varying susceptibility to the compounds. However, *Aspergillus niger* generally showed larger zones of inhibition at lower concentrations, possibly due to its thinner cell wall structure compared to other fungi. This made it more susceptible to the antifungal effects of the compounds**.** *Penicillium italicum*, with its more complex cell wall and resistance mechanisms, showed less sensitivity at the lower concentrations. The control treatment, with no compounds, did not produce any zone of inhibition, which confirms that the observed antifungal effects are directly attributable to the tested compounds. The results indicate that 2-hydroxy phenyl (3c) exhibited the strongest antifungal activity against both *Aspergillus niger* and *Penicillium italicum* across all concentrations tested, particularly at 10 mg. Other compounds like phenyl (3a)**,** 4-chloro phenyl (3b)**,** and 4-hydroxy 3-methoxy phenyl (3d) also showed varying degrees of antifungal activity, while 2-furyl (3e) showed moderate but promising results. Further studies are needed to explore the mechanisms of action of these compounds and to assess their potential for use in antifungal treatments.

Anti-inflammatory activity

All compounds showed a significant reduction in inflammation after 4 hours, with the most notable decrease observed in phenyl (3a), which exhibited the highest % inhibition of inflammation (71.80%). This suggests that phenyl (3a) has potent anti-inflammatory properties, more effective than the other synthesized compounds. The 4-chloro phenyl (3b) compound followed closely with an inhibition of 64.78%, indicating its strong anti-inflammatory activity, though slightly less effective than 3a.2-hydroxy phenyl (3c), 4-hydroxy 3-methoxy phenyl (3d), and 2-furyl (3e) also exhibited anti-inflammatory effects, with % inhibition values of 58.87%, 52.55%, and 49.87%, respectively. These compounds show moderate anti-inflammatory properties, suggesting that the presence of specific functional groups on the phenyl ring might be influencing the effectiveness of the compounds. The vehicle control group had the highest inflammation level at 4 hours (4.72±0.025), confirming that inflammation naturally progressed in the absence of anti-inflammatory agents. The standard (Indomethacin sodium) showed the highest reduction in inflammation (79.82% inhibition), as expected,

since indomethacin is a well-known anti-inflammatory agent. This sets a benchmark for comparing the efficacy of the synthesized compounds. The results suggest that the phenyl group (3a), in particular, is very effective in reducing inflammation. This could be due to the structural characteristics of the compound, which might enable it to interact effectively with inflammatory pathways or enzymes involved in the inflammatory process. The presence of additional functional groups like chloro, hydroxy, and methoxy in compounds like 4-chloro phenyl (3b) and 4-hydroxy 3-methoxy phenyl (3d) appears to modulate the anti-inflammatory activity, with 3b being notably more effective than 3d. This suggests that the nature of substitution on the phenyl ring may impact the compound's anti-inflammatory potential. Compounds like 2-furyl (3e), while still showing significant anti-inflammatory effects, were less potent than the others, indicating that the furan ring may be less effective than phenyl-based groups in this context. The data for phenyl (3a) and 4-chloro phenyl (3b) show statistically significant reductions in inflammation at 4 hours ($p < 0.05$, as indicated by **** and ** *), highlighting their stronger effects compared to other compounds. This study demonstrates that the synthesized compounds, particularly phenyl (3a), exhibit significant anti-inflammatory activity, with phenyl (3a) showing the highest inhibition of inflammation (71.80%). The presence of specific functional groups on the aromatic ring appears to influence the anti-inflammatory efficacy, with phenyl (3a) and 4-chlorophenyl (3b) being the most effective. Further research could explore the mechanisms through which these compounds exert their anti-inflammatory effects and whether they could be developed as alternative treatments to standard anti-inflammatory drugs like indomethacin.

Bronchodilator activity against histamine

Pheniramine maleate significantly increased the preconvulsion time (20.23 minutes), showing strong protection against convulsions (72.81% protection), as expected for a known antihistamine. Synthesized compound 3(a) also shows a significant effect, with a preconvulsion time of 16.35 minutes, providing 66.36% protection, though it's somewhat less effective than pheniramine. The control group had the shortest preconvulsion time (5.50 minutes) and no protection against histamine-induced convulsions. The significant p-value (<0.01) indicates that the results are unlikely due to chance and support the conclusion that both pheniramine and synthesized compound 3(a) are effective in protecting against histamine-induced convulsions. This shows that synthesized compounds of 1,3-dimethyl-7-(-3-phenyl prop-2-enoyl)-3,7-dihydro-1H-purine-2,6-dione 3(a) has antihistaminic property. Compound 3a showed an appreciable decrease in the severity of symptoms of asthma and also a simultaneous improvement in lung function parameters. Along with it has significant mast cell stabilizing activity and suggests significant bronchodilator activity against histamine.

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