RESEARCH PAPER

Synthesis and Biological Exploration of Novel Indeno[1,2-*d*]Pyrimidine-Based Heterocycles with Improved Antimicrobial Potential

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ABSTRACT

Background: Fused pyrimidine derivatives constitute an important class of heterocyclic compounds owing to their wide range of biological activities. Among them, indeno[1,2-*d*]-pyrimidinone derivatives have attracted considerable attention because of their promising antimicrobial, antifungal, antioxidant, and anticancer properties. In view of the growing demand for novel antimicrobial agents, the present study focused on the synthesis, characterization, and biological evaluation of a series of novel 2-amino-4-aryl-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives (**3a-f**).

Methodology: A series of substituted indeno[1,2-*d*]pyrimidinone derivatives (**3a-f**) were synthesized through a two-step synthetic route involving Knoevenagel condensation of 1,3-indandione with substituted aromatic aldehydes, followed by cyclocondensation with guanidine hydrochloride under reflux conditions. The synthesized compounds were purified by recrystallization and characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. Antimicrobial activity was evaluated against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Aspergillus fumigatus* and *Aspergillus niger* using the disc diffusion method at different concentrations, while ampicillin and clotrimazole served as reference drugs.

Results: The target compounds were obtained in good to excellent yields (85-92%) and their structures were successfully confirmed by spectroscopic and analytical techniques. Antimicrobial screening demonstrated that all synthesized derivatives exhibited concentration-dependent inhibitory activity against the tested microorganisms. Among the investigated compounds, the para-methyl-substituted derivative 3f displayed the highest antimicrobial activity, particularly against *S. mutans*, producing a zone of inhibition of 37 ± 5 mm at 100 µl concentration. The *para*-methoxy derivative **3d** also showed notable broad-spectrum activity. In contrast, derivatives containing electron-withdrawing substituents such as bromo, chloro, fluoro and nitro groups exhibited moderate antimicrobial effects. Structure–activity relationship analysis suggested that electron-donating substituents on the aryl ring enhanced the antimicrobial potential of the indeno[1,2-*d*]pyrimidinone scaffold.

Conclusion: The synthesized indeno[1,2-*d*]pyrimidinone derivatives represent a promising class of fused heterocyclic compounds with appreciable antimicrobial properties. The superior activity of the *para*-methyl and *para*-methoxy derivatives highlights the significance of electronic and lipophilic effects in modulating biological activity. These findings demonstrate that the indeno[1,2-*d*]pyrimidine framework is a valuable scaffold for the development of new antimicrobial agents and merits further investigation through detailed pharmacological and mechanistic studies.

Keywords: 5 keywords comma seperated

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INTRODUCTION

Nitrogen-containing heterocyclic compounds occupy a central position in medicinal chemistry because of their

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broad spectrum of biological and pharmaceutical applications. Among these heterocyclic systems, pyrimidine derivatives have attracted considerable attention owing to their structural resemblance to naturally occurring nucleic acid bases and their diverse pharmacological activities [1]. Pyrimidine-containing compounds exhibit antimicrobial, anticancer, antiviral, anti-inflammatory, antioxidant and antitubercular properties, making them important structural motifs in contemporary drug discovery and development [2,3].

Fusion of pyrimidine rings with substituted carbocyclic or heterocyclic systems often enhances molecular rigidity, lipophilicity and biological selectivity, thereby improving interaction with biological targets [4]. In this context, fused pyrimidine scaffolds, particularly indeno[1,2-*d*]pyrimidine derivatives, have emerged as promising bioactive molecules due to their significant anti-fungal, antimicrobial, antioxidant and anticancer activities [5,6]. The biological efficiency of these fused systems is largely associated with the presence of electron-rich nitrogen atoms, extended □-conjugation and a planar fused-ring framework, which facilitate stronger binding interactions with microbial enzymes, proteins and nucleic acids [7]. Moreover, substitution on the aromatic ring with electron-donating or electron withdrawing groups can considerably influence the pharmacological, lipophilicity behaviour and electronic distribution of pyrimidine derivatives, thereby affecting their biological performance [8].

The increasing emergence of microbial resistance toward existing antibiotics has created an urgent demand for the development of novel heterocyclic compounds with enhanced antimicrobial potential [9]. Fused pyrimidine derivatives have demonstrated promising activity against both Gram-positive and Gram-negative bacterial strains as well as pathogenic fungi through inhibition of microbial enzymes and interference with nucleic acid biosynthesis [10]. In addition to antimicrobial activities, these compounds also exhibit antioxidant activity by scavenging reactive oxygen species and interrupting oxidative chain reactions associated with cellular damage and chronic diseases such as cardiovascular cancer and diabetes disorders [11]. These observations highlight the pharmaceutical significance of developing structurally modified fused pyrimidine derivatives with improved biological profiles.

Indeno[1,2-*d*]pyrimidine derivatives have emerged as a prominent class of fused heterocyclic compounds in medicinal chemistry owing to their diverse biological activities, particularly their promising anticancer potential. These molecules have attracted considerable attention due to their ability to modulate key cellular pathways involved in cancer cell proliferation, survival, and progression [12]. The fusion of a pyrimidine nucleus with the indene scaffold affords structurally rigid and pharmacologically significant frameworks capable of interacting with a variety of biological targets associated with tumor development [13]. Among the various synthetic methodologies employed for the construction of fused pyrimidine systems, multicomponent condensation reactions have proven to be particularly advantageous due to their operational simplicity, high atom economy, and efficiency in generating structurally diverse heterocyclic architectures in a single synthetic step [14].

Motivated by the promising pharmacological profile of fused pyrimidine derivatives, the design and synthesis of novel indeno[1,2-*d*]pyrimidine analogs have become an active area of research. Structural modification of this scaffold offers opportunities to improve potency, selectivity, and pharmacokinetic properties, thereby facilitating the discovery of new anticancer candidates. Furthermore, molecular modeling and computational studies provide valuable insights into the structure–activity relationships and binding interactions of these compounds with relevant biological targets.

Notably, the incorporation of amino acid–derived or amino-functionalized substituents within such fused heterocyclic frameworks plays a significant role in enhancing biological performance [15]. Amino acid motifs can improve molecular recognition, increase hydrogen-bonding interactions with biological targets and enhance solubility and biocompatibility. When integrated into indeno[1,2-*d*]pyrimidine scaffolds, these functionalities may further modulate electronic distribution and strengthen ligand–target interactions, thereby contributing to improved antimicrobial and antioxidant efficacy.

In the present investigation, a series of novel indeno[1,2-*d*]pyrimidine derivatives (**3a-f**) were synthesized *via* cyclocondensation of substituted aromatic aldehydes, 1*H*-indene-1,3(2*H*)-dione and guanidine hydrochloride under acidic reflux conditions. In this transformation, guanidine hydrochloride plays a crucial role as a key cyclizing and nitrogen-donating reagent, facilitating the construction of the pyrimidine ring system. The reaction proceeds *via* an initial Knoevenagel condensation between the aromatic aldehyde and 1*H*-indene-1,3(2*H*)-dione, followed by Michael addition of guanidine and subsequent intramolecular cyclization leading to the formation of the fused indeno[1,2-*d*]pyrimidine scaffold [16]. Conventional reflux conditions are commonly employed due to their operational simplicity, reproducibility, and suitability for assembling complex heterocyclic frameworks. The synthesized compounds were characterized using FTIR ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. Furthermore, their biological potential was evaluated

through antimicrobial and antioxidant studies. The introduction of various substituted aromatic moieties into the fused pyrimidine framework is expected to enhance biological activity through synergistic structural effects.

MATERIALS AND METHODS

All reagents and solvents were purchased from commercial suppliers and used as received without further purification. Melting points were determined using a Gallenkamp melting point apparatus and are reported uncorrected. Infrared (IR) spectra were recorded on a Shimadzu FT-IR 8101 PC spectrophoto-meter using KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained in $CDCl_3$ or DMSO-d_6 and chemical shifts (□) are reported in parts per million (ppm) relative to the corresponding solvent signals. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer operating at 70 eV. Elemental analyses were performed using an Elementar Vario EL analyzer. Reaction progress and product purity were monitored by thin-layer chromato-graphy (TLC) on silica gel 60 F_{254} plates. Visualization was carried out under ultraviolet light where appropriate.

General Procedure for the synthesis of 2-amino-4-aryl-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives (3a-f): A mixture of 1,3-indandione (**1**, 1.46 g, 10 mmol) and the appropriate substituted aromatic aldehyde (**2a-f**, 10 mmol) was dissolved in absolute ethanol (25 mL) and piperidine (0.5 mL) was added as a catalyst. The react-ion mixture was heated under reflux (78 ºC) for 2-3 h with continuous stirring. The progress of the reaction was monitored by TLC using ethyl acetate/hexane (3:7, v/v) as the mobile phase. Upon completion, the reaction mix-ture was cooled to room temperature, and the resulting precipitate was collected by filtration, washed with cold ethanol, and dried to afford the corresponding arylidene-indane-1,3-dione intermediates (2a-f). Without further purification, the obtained intermediate (10 mmol) was suspended in absolute ethanol (30 mL), followed by the addition of guanidine hydrochloride (0.96 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol). The reaction mixture was refluxed for 5-7 h under constant stirring. Reaction progress was monitored by TLC using ethyl acetate/hexane (4:6, v/v). After completion, the reaction mixture was allowed to cool to ambient temperature and poured into crushed ice-water (50 mL). The resulting solid was filtered, washed thoro-ughly with distilled water to remove residual inorganic salts, and air-dried. The crude product was purified by recrystallization from ethanol to furnish the desired 2-amino-4-aryl-5*H*-indeno[1,2-d]pyrimidin-5-one derivatives (**3a-f**) as pure solids (**Scheme-I**). The synthesized compounds were obtained in good to excellent yields (72-90%) and exhibited purity greater than 95% as determined by TLC and elemental analysis. The structures of all synthesized derivatives were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis.

2-**Amino-4-(4-bromophenyl)-1,2,3,4-tetrahydro-5H-indeno[1,2-*d*]pyrimidin-5-one (3a):** Yield: 88%; colour: pale yellow solid; m.p.: 278-280 ºC. IR (KBr, ν_{max}, cm^{-1}): 3422, 3328 (NH_2), 3194 (NH), 1698 (C=O), 1614 (C=N), 1582 (Ar-C=C), 1268 (C–N), 755 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 4.82 (s, 1H, CH), 5.88 (br s, 2H, NH_2), 7.08-7.82 (m, 8H, Ar-H), 8.71 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 48.4, 113.1, 118.7, 122.9, 126.8, 128.3, 129.5, 131.8, 133.6, 141.7, 151.4, 160.9, 168.8. MS (*m/z*): 340 $[M+H]^+$, 342 $[M+H+2]^+$. Anal. calcd. for $C_{17}H_{14}BrN_3O$: C, 60.02; H, 4.15; N, 12.35%. Found: C, 59.88; H, 4.10; N, 12.28%.

2-Amino-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-5H-indeno[1,2-*d*]pyrimidin-5-one (3b): Yield: 90%; colour: cream solid; m.p.: 270-272 ºC; IR (KBr, ν_{max}, cm^{-1}): 3418, 3324, 3190, 1695, 1612, 1580, 1265, 782. ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 4.80 (s, 1H), 5.85 (br s, 2H), 7.05-7.78 (m, 8H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 48.1, 112.9, 118.5, 122.7, 126.5, 128.0, 129.2, 131.5, 134.4, 141.2, 151.1, 160.6, 168.5. MS (*m/z*): 296 $[M+H]^+$, 298 $[M+H+2]^+$. Anal. calcd. for $C_{17}H_{14}ClN_3O$: C, 68.58; H, 4.74; N, 14.11%. Found: C, 68.42; H, 4.69; N, 14.03%.

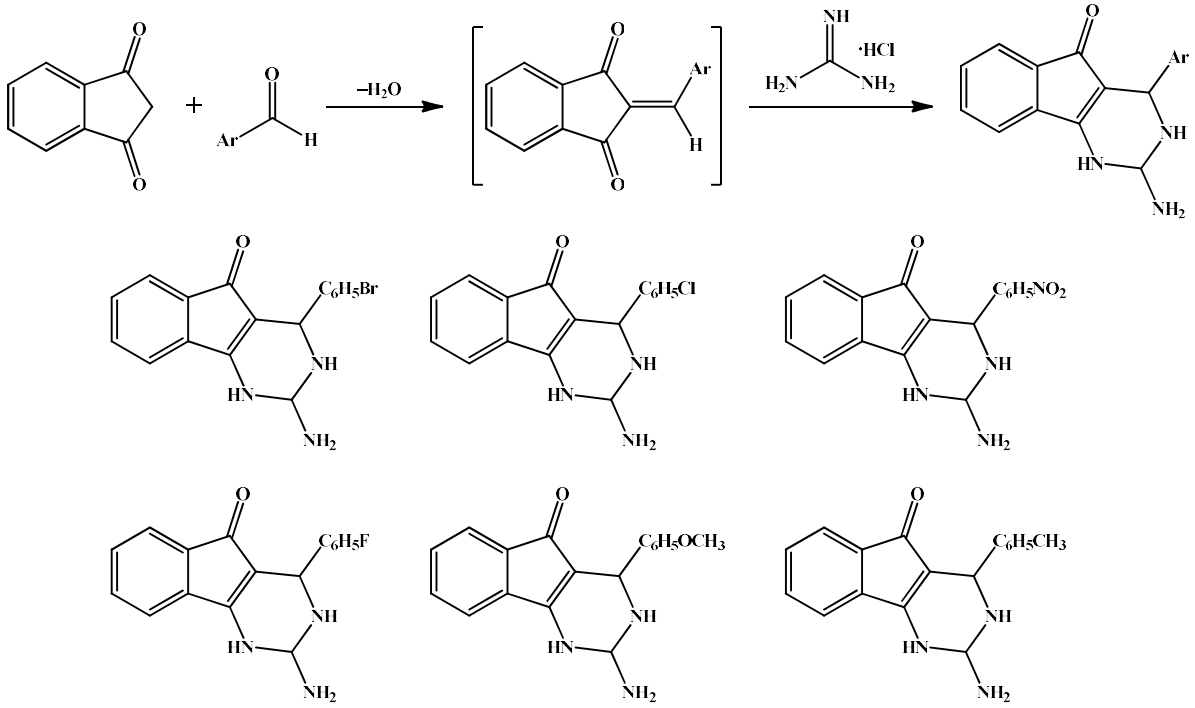
2-Amino-4-(4-fluorophenyl)-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one (5c): Yield: 92%; colour: off-white solid; m.p.: 262-264 ºC; IR (KBr, ν_{max}, cm^{-1}): 3425, 3330, 3192, 1697, 1613, 1585, 1220, 1267; ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 4.78 (s, 1H), 5.86 (br s, 2H), 7.02–7.76 (m, 8H), 8.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 48.0, 113.4, 115.6, 118.3, 122.5, 126.4, 128.1, 130.7, 140.9, 151.2, 160.5, 162.8, 168.3. MS (*m/z*): 280 $[M+H]^+$. Anal. calcd. for $C_{17}H_{14}FN_3O$: C, 73.11; H, 5.05; N, 15.04%. Found: C, 72.95; H, 4.99; N, 14.92%.

2-Amino-4-(4-methoxyphenyl)-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one (5d): Yield: 89%; colour: light yellow solid; m.p.: 248-250 ºC. IR (KBr, ν_{max}, cm^{-1}): 3420, 3325, 3188, 1694, 1610, 1515, 1248, 1032; ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 3.74 (s, 3H, OCH_3), 4.76 (s, 1H), 5.82 (br s, 2H), 6.88-7.74 (m, 8H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 48.2, 55.4, 113.8, 118.2, 122.4, 126.3, 127.8, 129.1, 140.6, 151.0, 158.9, 160.4, 168.2. MS (*m/z*): 292 $[M+H]^+$. Anal. calcd. for $C_{18}H_{17}N_3O_2$: C, 71.75; H, 5.69; N, 13.95%. Found: C, 71.61; H, 5.60; N, 13.82%.

2-Amino-4-(4-nitrophenyl)-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one (5e): Yield: 85%; colour: deep yellow solid; m.p.: 285-287 ºC; IR (KBr, ν_{max}, cm^{-1}):

vmax 3424, 3332, 3196, 1699, 1616, 1524, 1346, 1588. ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 4.90 (s, 1H), 5.92 (br s, 2H), 7.18-8.25 (m, 8H), 8.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 48.7, 113.6, 119.0, 123.4, 124.8, 127.2, 129.8, 141.9, 147.6, 151.8, 160.9, 168.9. MS (m/z): 307 $[M+H]^+$. Anal. calcd. for $C_{17}H_{14}N_4O_3$: C, 66.66; H, 4.61; N, 18.29%. Found: C, 66.52; H, 4.55; N, 18.12%.

2-Amino-4-(*p*-tolyl)-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]-pyrimidin-5-one (5f): Yield: 91%; colour: white crystalline solid; m.p.: 238-240 ºC. IR (KBr, □$_{max}$, cm^{-1}): 3418, 3322, 3187, 1693, 1608, 1578, 2922; ^{1}H NMR (400 MHz, DMSO-d_6, □ ppm): 2.28 (s, 3H, CH_3), 4.74 (s, 1H), 5.80 (br s, 2H), 7.00-7.72 (m, 8H), 8.60 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, □ ppm): 21.3, 48.0, 113.0, 118.1, 122.3, 126.2, 127.9, 129.4, 136.8, 140.5, 150.9, 160.3, 168.1; MS (*m/z*): 276 $[M+H]^+$. Anal. calcd. for $C_{18}H_{17}N_3O$: C, 78.52; H, 6.22; N, 15.26%. Found: C, 78.38; H, 6.16; N, 15.12%.



Scheme-I: synthesis of Indeno[1,2-d]Pyrimidine-Based Heterocycles

Antimicrobial activity: The synthesized compounds (**3a-f**) were screened for their antimicrobial potential against selected microbial strains. Antibacterial activity was assessed using two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, along with one Gram-negative bacterium, *Escherichia coli*. Antifungal efficacy was evaluated against *Candida albicans* and *Aspergillus* flavus. Each synthesized compound was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution with a concentration of 1 mg/mL. Sterile discs prepared from Whatman filter paper were cut to a uniform size and sterilized by autoclaving before use. The sterile discs were impregnated with the respective test solutions and carefully placed on nutrient agar plates, which had been previously inoculated with the test microorganisms. The inoculated Petri plates were incubated at 36 ºC for 24 h. Following incubation, the antimicrobial activity was determined by measuring the diameter of the inhibition zones surrounding each disc. All experiments were conducted in triplicate to ensure reproducibility of the results. For comparison, the antibacterial activity of ampicillin and the antifungal activity of clotrimazole as standards were evaluated under identical experimental conditions, using the same solvent system and concentration as the test compounds. The percentage activity index of each compound was subsequently calculated using the standard equation provided below:

RESULTS AND DISCUSSION

A series of novel 2-amino-4-aryl-5*H*-indeno[1,2-*d*]-pyrimidin-5-one derivatives (**3a-f**) were synthesized through a convenient two-step synthetic protocol involving a Knoevenagel condensation followed by cyclization with guanidine hydrochloride. Initially, 1,3-indandione (**1**) was condensed with appropriately substituted aromatic aldehydes (**2a-f**) in ethanol using piperidine as a base catalyst to afford the corresponding 2-arylidene-indane-1,3-dione intermediates. The reaction proceeds through deprotonation of the active methylene group of 1,3-indandione followed by nucleophilic addition to the

aldehyde carbonyl and subsequent dehydration, yielding the conjugated □,□-unsaturated diketone intermediates (**Scheme-I**). In the second step, the arylidene-indane-1,3-dione intermediates underwent cyclocondensation with guanidine hydrochloride in the presence of anhydrous potassium carbonate under reflux conditions. Potassium carbonate acts as a base to liberate free guanidine, which subsequently attacks the electrophilic carbonyl center of the intermediate. Intramolecular cyclization followed by dehydration furnished the target 2-amino-4-aryl-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives (**3a-f**). The synthesized compounds were obtained in good to excellent yields (85-92%), indicating the efficiency of the adopted synthetic strategy.

The structures of all the synthesized compounds **3a-f** were established through FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. The IR spectra of all synthesized derivatives displayed characteristic absorption bands corresponding to the indeno[1,2-*d*]pyrimidinone framework. Broad absorption bands observed in the region 3425-3418 cm^{-1} and 3332-3322 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations of the amino (NH_2) group. A distinct absorption band around 3196-3187 cm^{-1} confirmed the presence of the NH functionality within the pyrimidine ring. The strong absorption observed between 1699-1693 cm^{-1} was attributed to the carbonyl (C=O) group of the pyrimidinone moiety, while bands appearing at 1616-1608 cm^{-1} indicated the presence of azomethine (C=N) stretching vibrations. The aromatic skeletal vibrations appeared within 1588-1578 cm^{-1}. Additional substituent-specific absorptions were observed, including C–Br (755 cm^{-1}) for compound **3a**, C–Cl (782 cm^{-1}) for **3b**, C–F (1220 cm^{-1}) for **3c**, C–O–C stretching (1248 and 1032 cm^{-1}) for **3d**, NO_2 asymmetric and symmetric stretching (1524 and 1346 cm^{-1}) for **3e** and aliphatic C–H stretching (2922 cm^{-1}) for **3f**.

The ^{1}H NMR spectra of compounds **3a-f** exhibited a characteristic singlet corresponding to the methine proton (CH) at the C-4 position of the pyrimidine ring within the range □ 4.74-4.90 ppm. The amino protons appeared as broad singlets at □ 5.80-5.92 ppm, confirming the presence of the 2-amino substituent. The aromatic protons of the fused indene and substituted phenyl rings resonated as multiplets between □ 6.88-8.25 ppm depending on the electronic nature of the substituent. The NH proton of the pyrimidinone ring consistently appeared as a singlet in the downfield region at □ 8.60-8.80 ppm, indicating involvement in hydrogen bonding. Compound **3d** exhibited an additional singlet at □ 3.74 ppm corresponding to the methoxy (OCH_3) protons, whereas compound **3f** displayed a singlet at □ 2.28 ppm due to the methyl (CH_3) substituent.

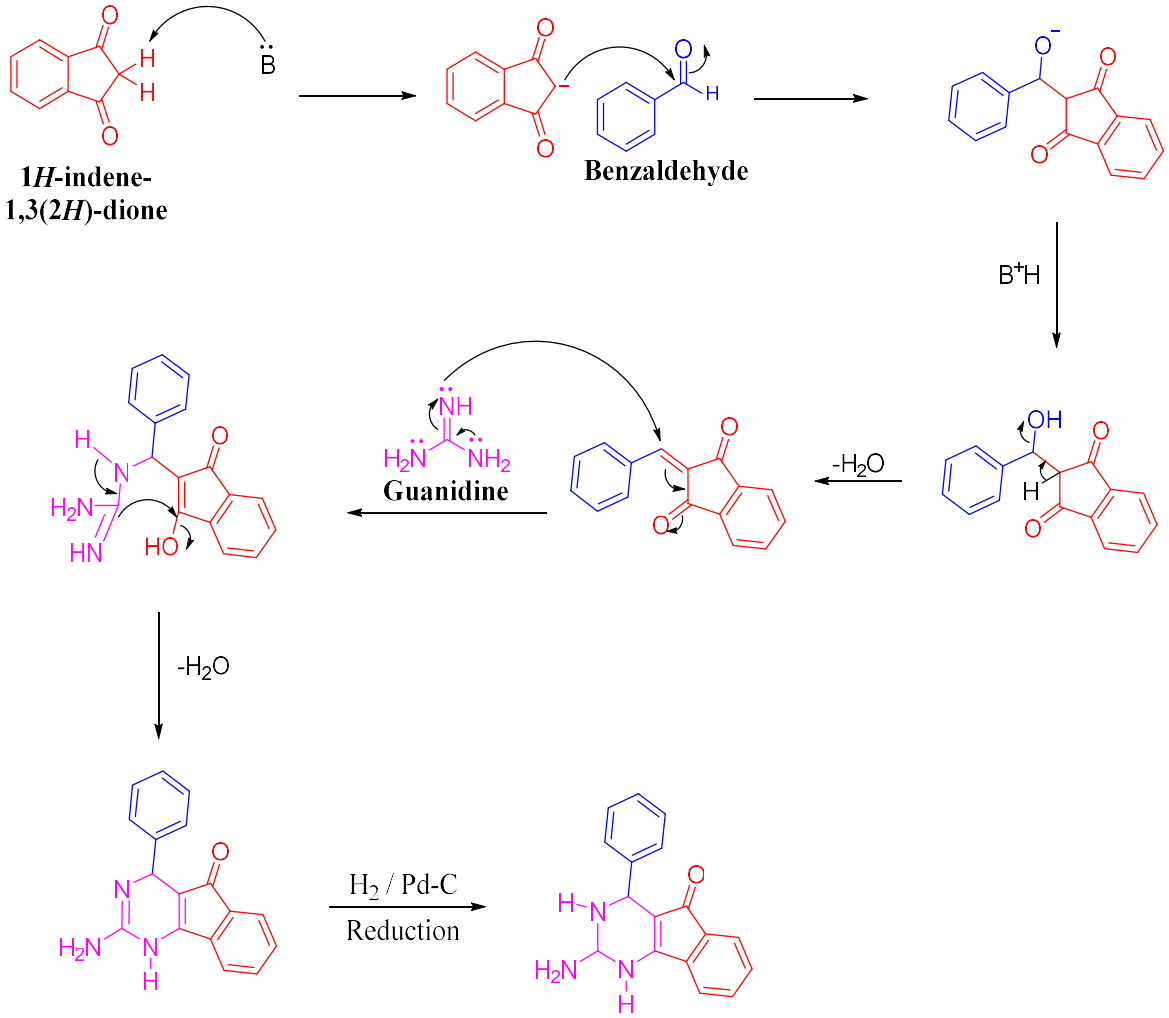
The ^{13}C NMR spectra further supported the assigned structures. The methine carbon attached to the pyrimidine ring resonated at approximately □ 48.0-48.7 ppm in all compounds. The carbonyl carbon of the pyrimidinone moiety appeared in the downfield region at □ 168.1-168.9 ppm, confirming the presence of the ketonic functionality. The signals observed between □ 150.9-162.8 ppm were assigned to the pyrimidine C=N carbons and substituted aromatic carbons attached to heteroatoms. The aromatic carbons of the indene and phenyl rings resonated within □ 112-142 ppm. Compound **3d** showed a characteristic methoxy carbon signal at □ 55.4 ppm, while compound **3f** displayed a methyl carbon resonance at □ 21.3 ppm. The nitro-substituted derivative **3e** exhibited a relatively deshielded aromatic carbon signal at □ 147.6 ppm due to the strong electron-withdrawing effect of the nitro group.

Mass spectrometric studies further confirmed the molecular structures of the synthesized derivatives. Compound **3a** displayed molecular ion peaks at *m/z* 340 and 342 $[M+H]^+$, corresponding to the characteristic isotopic pattern of bromine. Similarly, compound **3b** exhibited peaks at *m/z* 296 and 298 $[M+H]^+$, confirming the presence of chlorine. Compounds **3c-f** showed molecular ion peaks at *m/z* 280, 292, 307 and 276 $[M+H]^+$, respectively, consistent with their calculated molecular weights.

Plausible mechanism: Based on the reaction scheme and spectra data, a plausible synthetic mechanism of 2-amino-4-aryl-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives (**3a-f**) proceeds through a sequential Knoevenagel condensation followed by guanidine-mediated cyclization (**Scheme-II**). Initially, piperidine abstracts the acidic methylene proton of 1,3-indandione, generating a resonance-stabilized enolate ion. The nucleophilic enolate attacks the carbonyl carbon of the substituted aromatic aldehyde to form a □-hydroxy intermediate, which subsequently undergoes base-assisted dehydration to afford the corresponding 2-arylidene-indane-1,3-dione intermediate. The formation of this conjugated □,□-unsaturated system is thermodynamically favoured due to extensive □-electron delocalization across the indandione framework.

In the second stage, guanidine hydrochloride is deprotonated by potassium carbonate to generate the free guanidine nucleophile. The amino nitrogen of guanidine performs a Michael-type nucleophilic addition to the electrophilic □-carbon of the arylidene-indane-1,3-dione intermediate, producing an open-chain adduct. Subsequent intramolecular nucleophilic attack of the second amino group on one of the carbonyl carbons of the indandione moiety leads to ring closure and formation of a dihydropyrimidine intermediate. Proton transfer and

tautomeric rearrangements stabilize the newly formed heterocyclic system, followed by the elimination of a molecule of water to generate the fused indeno[1,2-*d*]pyrimidinone skeleton. The initially formed product may exist in an imino-enamine tautomeric form, which undergoes further proton migration and reduction of the exocyclic double bond under the reaction conditions to furnish the thermodynamically stable 2-amino-4-aryl-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives.



2-amino-4-phenyl-1,2,3,4-tetrahydro-5*H*-indeno[1,2-*d*]pyrimidin-5-one

Scheme-II

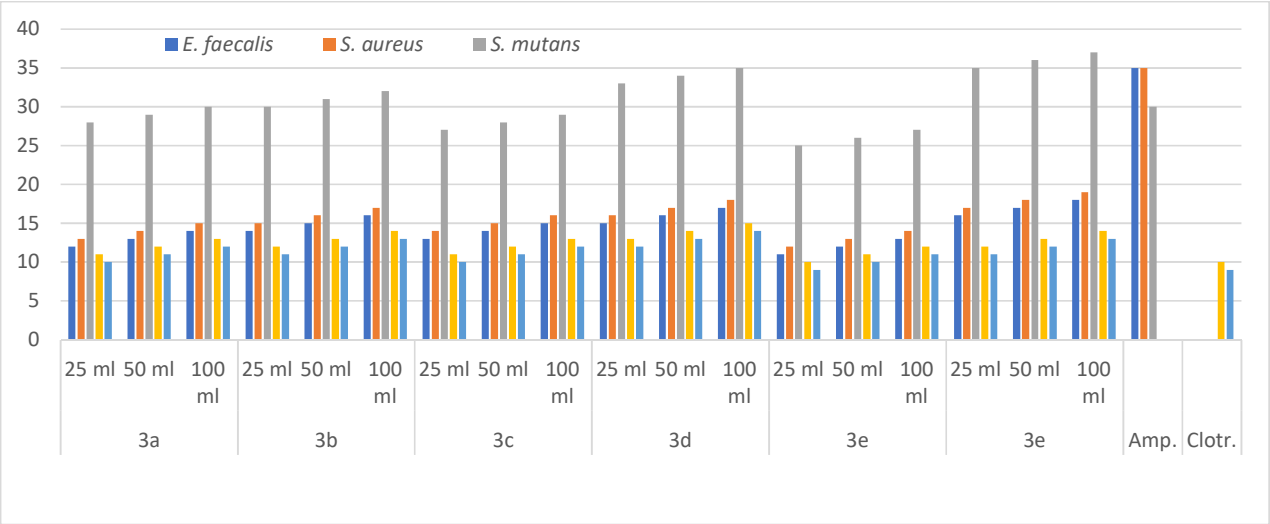


Figure 1

Antimicrobial activities: The antimicrobial activities of the synthesized 2-amino-4-aryl-5H-indeno[1,2-*d*]pyrimidin-5-one derivatives (**3a-f**) were evaluated against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Aspergillus fumigatus* and *Aspergillus niger* using the disc diffusion method at concentrations of 25, 50 and 100 □l. The results revealed a concentration-dependent increase in the zone of inhibition for all compounds. Among the tested microorganisms, *S. mutans* was found to be the most susceptible strain, whereas *A. niger* exhibited comparatively lower sensitivity toward the synthesized derivatives (Table-1).

Among the compounds investigated, **3f** (4-methyl substituted derivative) demonstrated the highest antimicrobial activity, producing inhibition zones of 18 ± 4 mm, 19 ± 4 mm, 37 ± 5 mm, 14 ± 3 mm, and 13 ± 3 mm against *E. faecalis*, *S. aureus*, *S. mutans*, *A. fumigatus* and *A. niger*, respectively, at 100 µl concentration. Compound **3d** (4-methoxy derivative) also exhibited significant activity with inhibition zones of 17 ± 4 mm, 18 ± 4 mm, 35 ± 5 mm, 15 ± 3 mm and 14 ± 3 mm against the respective microorganisms. The halogen-substituted derivatives 3**a-c** displayed moderate activity, while the nitro-substituted derivative **3e** showed comparatively lower antimicrobial efficacy than compounds **3d** and **3f** (Fig. 1, Table-1).

Table-1: Zone of inhibition (mean ± SD) of Indeno[1,2-d]pyrimidine derivatives (3a–3f) at different concentrations

Micro-organism	3a			3b			3c			3d			3e			3e			Ampicillin	Clotrimazole
	25 □l	50 □l	100 □l	25 □l	50 □l	100 □l	25 □l	50 □l	100 □l	25 □l	50 □l	100 □l	25 □l	50 □l	100 □l	25 □l	50 □l	100 □l		
E. faecalis	12 ±3	13 ±3	14 ±3	14 ±3	15 ±3	16 ±4	13 ±3	14 ±3	15 ±3	15 ±4	16 ±4	17 ±4	11 ±3	12 ±3	13 ±3	16 ±4	17 ±4	18 ±4	35±6	—
S. aureus	13 ±3	14 ±3	15 ±4	15 ±4	16 ±4	17 ±4	14 ±3	15 ±3	16 ±4	16 ±4	17 ±4	18 ±4	12 ±3	13 ±3	14 ±3	17 ±4	18 ±4	19 ±4	35±6	—
S. mutans	28 ±4	29 ±4	30 ±4	30 ±4	31 ±4	32 ±4	27 ±3	28 ±3	29 ±3	33 ±4	34 ±4	35 ±5	25 ±3	26 ±3	27 ±3	35 ±5	36 ±5	37 ±5	30±5	—
A. fumigatus	11 ±3	12 ±3	13 ±3	12 ±3	13 ±3	14 ±3	11 ±3	12 ±3	13 ±3	13 ±3	14 ±3	15 ±3	10 ±2	11 ±2	12 ±2	12 ±3	13 ±3	14 ±3	—	10±5
A. niger	10 ±3	11 ±3	12 ±3	11 ±3	12 ±3	13 ±3	10 ±3	11 ±3	12 ±3	12 ±3	13 ±3	14 ±3	9± 2	10 ±2	11 ±2	11 ±3	12 ±3	13 ±3	—	9±5

A comparison of the structure–activity relationship suggests that electron-donating substituents such as methyl and methoxy groups at the para position of the phenyl ring enhanced antimicrobial activity, particularly against *S. mutans*. In contrast, compounds bearing electron-withdrawing substituents (Br, Cl, F and NO_2) exhibited moderate inhibitory effects. Although the synthesized compounds showed lower activity than the reference antibiotic ampicillin against bacterial strains, compounds **3d** and **3f** displayed appreciable broad-spectrum antimicrobial properties. Similarly, their antifungal activities against *A. fumigatus* and *A. niger* were

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comparable to or slightly superior to the standard clotrimazole under the tested conditions. These findings indicate that *para*-substituted electron-donating groups favor antimicrobial potency in the indeno[1,2-*d*]pyrimidinone scaffold and provide useful leads for further optimization of this class of compounds.

CONCLUSION

In summary, a series of six novel 2-amino-4-aryl-5*H*-indeno[1,2-*d*]pyrimidin-5-one derivatives (**3a-f**) were successfully synthesized via an efficient and reproducible two-step protocol involving Knoevenagel condensation and guanidine-mediated cyclization. The synthetic methodology afforded the target compounds in high yields with excellent purity. Comprehensive spectroscopic and analytical investigations, including FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis, unequivocally established the structures of the synthesized molecules. Antimicrobial evaluation demonstrated that all derivatives possess measurable antibacterial and antifungal activities, with activity generally increasing with concentration. Compounds **3f** and **3d** emerged as the most active analogues, particularly against *Streptococcus mutans*, suggesting that electron-donating substituents on the aromatic ring contribute positively to antimicrobial efficacy. The observed structure–activity relationship provides useful insights for future molecular modifications aimed at improving biological performance. Based on the findings, the present findings demonstrate that indeno[1,2-*d*]pyrimidinone derivatives represent promising heterocyclic scaffolds for the development of new antimicrobial agents and warrant further investigation, including mechanistic studies, molecular docking analyses and *in vivo* biological evaluation.

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