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Synthesis and biological evaluation of an indanone-pyrazoline hybrid compound and its synthetic intermediates

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ABSTRACT

Pyrazoline and indanone scaffolds are two important skeletal motifs widely used in medicinal chemistry for their broad biological activities. In the current investigation, a novel indanone-pyrazoline hybrid compound was prepared in a three-step synthetic sequence. Synthesis consisted of the formation of a Schiff base via reaction with indanone, Claisen-Schmidt condensation to obtain the indanone chalcone, and phenylhydrazine cyclization to yield the desired pyrazoline. The synthesized compounds were evaluated using ¹H NMR spectroscopy, while ¹³C NMR data were additionally obtained for compounds 1 and 3. Antimicrobial testing using the agar diffusion test produced inhibition zones of 11–12 mm against *E. coli* and *S. aureus*. The hybrid compound showed a 12 mm inhibition zone, whereas the intermediates produced an 11 mm inhibition zone; however, the biological relevance of this small numerical difference should be interpreted cautiously. For antifungal activity, *C. albicans* showed inhibition zones of 10–11 mm, with the Schiff base intermediate producing a slightly larger inhibition zone than the hybrid compound. At the same time, no inhibition was observed for *A. flavus*.

KEYWORDS

 Chalcone
 Indanone
 Pyrazoline
 Schiff base
 Antimicrobial activity
 Agar diffusion method
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1. Introduction

Heterocyclic compounds are of great interest to organic and medicinal chemists due to their structural diversity and wide range of biological activities. Among nitrogen-containing heterocycles, pyrazolines have attracted considerable attention because of their broad pharmacological activities and synthetic accessibility. Pyrazolines are commonly synthesized by the cyclocondensation of α,β -unsaturated carbonyl compounds, particularly chalcones, with hydrazine derivatives [1,2]. Pyrazolines may exist as 1-, 2-, or 3-pyrazoline isomers [2].

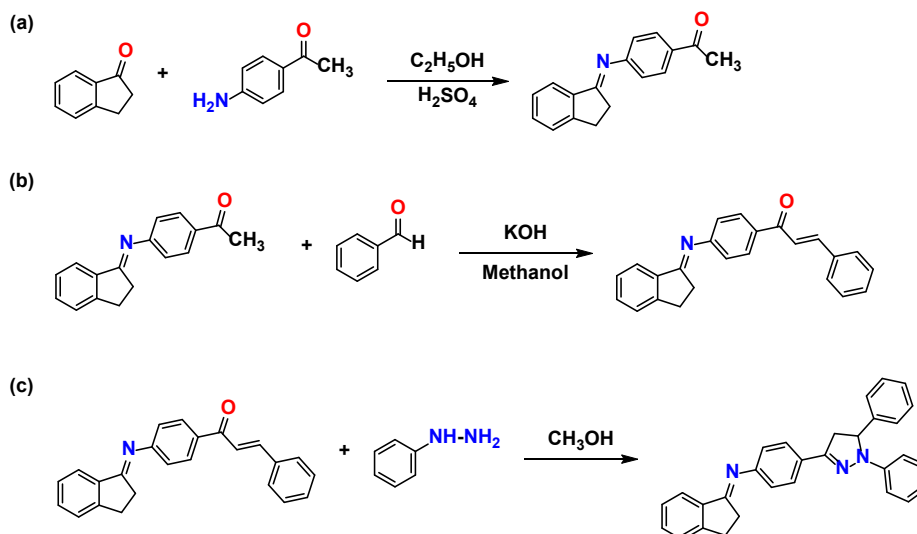
Pyrazoline derivatives have been reported to exhibit diverse biological activities, including antimicrobial, anti-inflammatory, and anticancer effects [1,2]. Thiazolone-based compounds containing a pyrazoline moiety have also demonstrated *in vitro* anticancer activity against several human cancer cell lines [3]. Certain 2-pyrazoline derivatives bearing a benzene sulfonamide moiety have also been evaluated for anti-inflammatory and anticancer activities [4].

Chalcones are another important class of organic molecules that are commonly used as intermediates in heterocyclic synthesis. Chemically defined as 1,3-diphenyl-2-propen-1-ones, chalcones contain an electrophilic α , β -unsaturated carbonyl system that contributes to their high reactivity and

broad pharmacological potential. Numerous chalcone derivatives have previously been shown to possess a variety of pharmacological activities, including antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer properties [5]. Chalcones are commonly synthesized through Claisen-Schmidt condensation [6] and subsequently transformed into heterocyclic compounds [7].

Indanone is a privileged scaffold in medicinal chemistry. It is present in several biologically active molecules and can be prepared through oxidation of indane/indene derivatives or cyclization strategies. Substituted indanone derivatives have been investigated for applications including enzyme inhibition and neuroprotective drug discovery. Several substituted 1-indanone derivatives have been investigated as potential inhibitors of acetylcholinesterase for use in the treatment of Alzheimer's disease [8]. Recent annulation and cyclization strategies involving 1-indanones have broadened access to fused and spiro frameworks [9].

Molecular hybridization combines pharmacophoric moieties from different bioactive compounds within a single molecular framework and is widely used as a strategy in drug design [10]. Reviews have also highlighted the role of molecular hybridization in the design of anticancer agents [11].



Scheme 1. Synthesis of indanone-pyrazoline hybrid compounds.

Although the pharmacological significance of the indanone and pyrazoline moieties is well recognized, the synthesis of hybrid systems comprising both substructures has been rather scarce. With increasing demand for new bioactive compounds and the limitations of current medicinal agents, the investigation of novel nitrogen-heterocyclic systems remains a key target. Hence, the current investigation was designed to synthesize a hybrid indanone-pyrazoline compound via a multi-step synthesis, including the preparation of an indanone-based Schiff base and an indanone-chalcone, followed by cyclization with phenylhydrazine. The compounds were characterized by spectral analysis and screened for preliminary antimicrobial activity using the agar diffusion method. This work presents an initial evaluation of the indanone-pyrazoline motif; subsequent synthesis of a larger series of derivatives is needed for detailed SAR.

2. Experimental

2.1. Materials

The USKT laboratory supplied chemicals consisting of indanone and phenylhydrazine. Potassium hydroxide (KOH), ethanol (EtOH), methanol (MeOH), and sulphuric acid (H_2SO_4) were purchased from Sigma-Aldrich and Multi Tech. Additional reagents, including ethyl acetate, *n*-hexane, dichloromethane (DCM), and 4-aminoacetophenone were obtained from the USKT lab.

2.2. Instrumentation

Melting points were determined using a DMP-800 instrument in capillary tubes. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers (operating at 400 MHz for ^1H and 100 MHz for ^{13}C) using $\text{DMSO}-d_6/\text{CDCl}_3$ as the solvent and tetramethylsilane (TMS) as an internal reference. Other laboratory equipment included drying ovens, vacuum dryers, measuring balances, and rotary evaporators.

2.3. General procedure

All reactions were monitored by thin-layer chromatography (TLC) using silica gel plates, and spots were visualized under UV light. Reaction progress and completion were

determined based on disappearance of starting material. The solid products were isolated by filtration and dried.

2.4. Synthesis of indanone-pyrazoline hybrid compound

Target indanone-pyrazoline hybrid compound was obtained through a three-step synthesis. In the first step, an indanone-based Schiff base was prepared, and in the second step, it was converted into indanone-chalcone intermediates. The last step was the cyclization of indanone-chalcone intermediates to give indanone-pyrazoline hybrid compound.

2.4.1. Synthesis of 1-(4-((2,3-dihydro-1H-inden-1-ylidene)amino)phenyl)ethan-1-one

Indanone (1.00 g, 0.007566 mol) and 4-aminoacetophenone (1.02 g, 0.007546 mol) were dissolved in ethanol (15 mL) in a 250 mL flask. A few drops of concentrated sulfuric acid were added as a catalyst, and the reaction mixture was stirred at room temperature for 6 h. The progress of the reaction was monitored periodically by thin-layer chromatography (TLC) using a mixture of ethyl acetate/*n*-hexane (1:99) solvent system. When the reaction was complete, the reaction mixture was filtered, and the solid product was collected. The solid product was vacuum-dried for 7 h to give the Schiff base (Scheme 1).

1-(4-((2, 3-Dihydro-1H-inden-1-ylidene)amino)phenyl)ethan-1-one: Color: Off-white. Yield: 90%. *R*_f: 0.54. M.p.: 220–222 °C. ^1H NMR (400 MHz, CDCl_3 , δ , ppm): 8.06 (d, 2H, Ar-H), 7.48 (d, 1H, Ar-H), 7.37–7.26 (m, 4H, Ar-H), 7.17 (d, 1H, Ar-H), 3.08 (t, 2H, CH_2), 2.82 (t, 2H, CH_2), 2.50 (s, 3H, COCH_3). ^{13}C NMR (100 MHz, CDCl_3 , δ , ppm): 195.81 (C=O), 165.44 (C=N), 164.46, 156.83, 148.54, 133.63, 133.36, 133.20, 129.95, 127.25, 126.02, 122.92 (Ar-C), 29.03 ($\text{CH}_2\text{-CH}_2$), 24.07 (CH_3).

2.4.2. Synthesis of 1-(4-((2,3-dihydro-1H-inden-1-ylidene)amino)phenyl)-3-phenylprop-2-en-1-one

The previously synthesized Schiff base (1.20 g, 0.0048 mol) was dissolved in methanol (30 mL) in a 250 mL round-bottom flask. Potassium hydroxide (0.86 g) was added to the reaction mixture, which was then heated at 50–60 °C with continuous stirring for approximately 20 minutes until the solids were completely dissolved. Subsequently, benzaldehyde (1.4 mL) was added dropwise, and the reaction mixture was stirred for

12 hours under the same temperature conditions. The progress of the reaction was monitored by thin-layer chromatography (TLC) using an ethyl acetate/*n*-hexane (1:99) solvent system. The precipitated product was collected by filtration. The precipitate was then dried in a vacuum oven for about 6–7 h to produce the intermediate compound indanone-chalcone (yellow crystalline solid) (Scheme 1).

1-(4-((2, 3-Dihydro-1H-inden-1-ylidene)amino)phenyl)-3-phenylprop-2-en-1-one: Color: Yellow. Yield: 87%. *R*_f: 0.72. M.p.: 68–70 °C. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 8.07 (d, 1H, Ar-H), 7.86–7.76 (m, 4H, Ar-H), 7.63–7.28 (m, 9H, Ar-H+CH=CH), 7.19 (d, 1H, CH=CH), 3.07 (t, 2H, CH₂), 2.87 (t, 2H, CH₂).

2.4.3. Synthesis of *N*-(2,3-dihydro-1H-inden-1-ylidene)-4-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)aniline

The indanone-chalcone intermediate (0.38 g, 0.001186 mol) was dissolved in methanol (15–20 mL) in a 250 mL round-bottomed flask equipped with a condenser. The solution was stirred continuously and heated under reflux for 20–25 minutes until the solid had almost dissolved. Phenylhydrazine (0.12 mL) was then added dropwise to the reaction mixture which was then further refluxed for 15 h. The progress of the reaction was monitored throughout by thin-layer chromatography (TLC) analysis (on silica gel plate and the solvent system was ethyl acetate/*n*-hexane 3:97). After this period, the reaction mixture was cooled to room temperature and left to rest for 15–20 minutes. The solids were separated by filtration and dried at room temperature for about 4 hours (Scheme 1).

N-(2, 3-Dihydro-1H-inden-1-ylidene)-4-(1, 5-diphenyl-4, 5-dihydro-1H-pyrazol-3-yl)aniline: Color: Dark brown. Yield: 92%. *R*_f: 0.51. M.p.: 50–52 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 7.64–7.58 (m, Ar-H), 7.41–7.36 (m, Ar-H), 3.36–3.34 (m, CH₂). The expected pyrazoline methine (CH) resonance could not be unambiguously identified in the ¹H NMR spectrum under the measurement conditions used. ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 142.00 (C-N), 137.13, 133.13, 132.00, 131.61, 131.30, 130.71, 130.47, 130.26, 130.10, 128.89, 128.86, 128.47, 127.95, 127.32, 126.38, 126.18, 126.03, 125.06, 125.00, 124.58, 124.19, 123.23, 122.49 (Ar-C), 53.43 (CH), 45.98 (CH₂).

2.5. Antimicrobial activity

Antibacterial and antifungal activities of the newly synthesized Schiff base of indanone, indanone-chalcone intermediate, and hybrid compound indanone-pyrazoline were studied using the agar diffusion method. For antibacterial activity, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), which are Gram-negative and Gram-positive, respectively, were used; for antifungal activity, *Aspergillus flavus* (ATCC 204304) and *Candida albicans* (ATCC 10231) were used.

For the antibacterial assay, Mueller-Hinton agar (MHA) medium was employed. For the antifungal assay, Sabouraud dextrose agar (SDA) medium was utilized. Fresh cultures were prepared, and bacterial suspensions were prepared to yield a 0.5 McFarland turbidity standard, corresponding to about 1.5 × 10⁸ CFU/mL. The fungal suspensions were prepared under sterile conditions and standardized. Inoculation was performed on all prepared agar media using the respective microbial suspensions.

The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to give solutions containing the test compounds at a concentration of 20 mg/mL. Wells (6 mm in diameter) were prepared on the inoculated agar plates, and 100 μL of each test compound was put in each well. DMSO (100 μL) was used as the negative control in the experiment. Tetracycline was used as the positive control for antibacterial activity. Fluconazole and amphotericin B were used as positive controls for antifungal activity. The positive-control solutions

were applied at the same concentration (20 mg/mL) and volume (100 μL per well) as the test-compound solutions.

The plates inoculated with *E. coli* and *S. aureus* were incubated at 37 °C for 24 h. The *C. albicans* plates were incubated at 37 °C for 24–48 h, whereas the *A. flavus* plates were incubated at 28 °C for 48–72 h. Following incubation, antimicrobial activity was determined by measuring the diameter of the clear inhibition zones surrounding the wells in millimeters (mm), including the well diameter. The assay was treated as a preliminary screening, and no inferential statistical comparison was performed. All antimicrobial assays were performed in three independent experiments, and the inhibition-zone diameters are reported as mean ± standard deviation (n = 3).

3. Results and discussion

3.1. Synthesis and structural characterization of compounds 1–3

The synthesis of substituted pyrazoline, as shown in Scheme 1, was carried out in a consecutive three-step sequence starting from indanone and 4-aminoacetophenone. In the first step, indanone and 4-aminoacetophenone were reacted to form the corresponding Schiff base, Compound 1, which was isolated as an off-white precipitate in 90% yield. In the subsequent step, Compound 1 was subjected to Claisen–Schmidt condensation with benzaldehyde to afford the corresponding chalcone intermediate, Compound 2. Finally, Compound 2 was reacted with phenylhydrazine to yield the target indanone-pyrazoline, Compound 3, which was obtained as a dark brown precipitate in 92% yield.

The structure of the synthesized compound 1 (Scheme 1a) was confirmed by ¹H and ¹³C NMR spectroscopy. The ¹H NMR spectrum (400 MHz, CDCl₃) revealed characteristic aromatic proton signals occurring between δ 8.06 and 7.17 ppm. A doublet peak at δ 8.06 ppm, as well as the peaks at δ 7.48 ppm (d, 1H), 7.37–7.26 ppm (m, 4H), and 7.17 ppm (d, 1H), each correspond to aromatic protons. The peaks at δ 3.08 ppm (t, 2H) and 2.82 ppm (t, 2H) represent methylene (–CH₂–CH₂–) groups in the aliphatic region, while the singlet at 2.50 ppm (3H) depicts a methyl group (–COCH₃). The ¹³C NMR spectrum (100 MHz, CDCl₃) further supported the structure. The resonance at δ 195.81 ppm was assigned to the carbonyl carbon of the acetyl group. The signal at δ 165.44 ppm was attributed to the imine carbon (C=N), while the resonances between δ 164.46 and 122.92 ppm were associated with aromatic carbon environments. The aliphatic region appears at δ 29.03 ppm and corresponds to methylene carbons. The signal at δ 24.07 ppm was attributed to the methyl carbon of the acetyl group.

The ¹H NMR spectrum (400 MHz, CDCl₃) of compound 2 displayed many chemical shifts that were assigned to aromatic and vinylic protons. Aromatic protons in compound 2 were assigned to a resonance at δ 8.07 ppm (1H), a multiplet chemical shift from δ 7.86 to 7.76 ppm (4H), and a further multiplet from δ 7.63 to 7.28 ppm (9H). Furthermore, the chemical shifts of the vinylic protons (–CH=CH–) were a multiplet at δ 7.63 to 7.28 ppm and a doublet at δ 7.19 ppm (1H). The triplet at δ 3.07 ppm (2H) and δ 2.87 ppm (2H) indicated the presence of the methylene (–CH₂–CH₂–) protons.

The ¹H and ¹³C NMR spectral features of compound 3 (Scheme 1c) are discussed below. The resonances at δ 7.64–7.58 and 7.41–7.36 ppm are assigned to the aromatic protons of the aryl groups. The absence of residual vinylic (–CH=CH–) proton signals is compatible with cyclization of the chalcone precursor. A multiplet was observed at δ 3.36–3.34 ppm in the aliphatic region. However, the expected pyrazoline methine (CH) resonance was not clearly observed under the measurement conditions.

Table 1. Inhibition-zone diameters of the tested compounds and controls (mean $\pm$ SD, mm; n = 3).

Compound	<i>E. coli</i>	<i>S. aureus</i>	<i>A. flavus</i>	<i>C. albicans</i>
Indanone-4-aminoacetophenone Schiff base	11 $\pm$ 0.58	11 $\pm$ 0.58	0	11 $\pm$ 0.58
Indanone-chalcone intermediate	11 $\pm$ 0.58	11 $\pm$ 0.58	0	10 $\pm$ 0.58
Indanone-pyrazoline hybrid compound	12 $\pm$ 0.58	12 $\pm$ 0.58	0	10 $\pm$ 0.58
Tetracycline	32 $\pm$ 1.00	34 $\pm$ 1.00	-	-
Fluconazole	-	-	14 $\pm$ 1.00	16 $\pm$ 1.00
Amphotericin B	-	-	16 $\pm$ 1.00	20 $\pm$ 1.00

* 0 = No inhibition; - = not tested.

The ^{13}C NMR spectrum (100 MHz, CDCl_3) displays resonances at δ 53.43 and 45.98 ppm, attributable to aliphatic carbon environments, together with multiple signals between δ 137.13 and 122.49 ppm corresponding to aromatic carbons. Although the resonance at δ 53.43 ppm is attributable to an aliphatic carbon environment, the corresponding methine proton was not clearly observed in the ^1H NMR spectrum.

3.2. Antimicrobial activity

The antimicrobial activity of the synthesized indanone-based Schiff base, indanone-chalcone intermediate, and indanone-pyrazoline hybrid compound was tested on *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus*, and *Candida albicans*. Zones of inhibition observed are given in Table 1.

Both the indanone-based Schiff base and the indanone-chalcone intermediate produced inhibition zones of 11 mm against *E. coli* and *S. aureus*. Specifically, both compounds showed an 11 mm zone of inhibition against both bacteria, while the indanone-pyrazoline hybrid compound showed a slightly higher zone of 12 mm. However, this 1 mm numerical difference should not be interpreted as evidence of superior antimicrobial activity.

No inhibition was observed against *A. flavus* with any of the synthesized compounds. In contrast, inhibition was observed against *C. albicans*, with inhibition zones ranging from 10 to 11 mm. Indanone-derived Schiff base gave an 11 mm inhibition zone, while indanone-chalcone derivative and indanone-pyrazoline derivatives each gave a 10 mm inhibition zone. Thus, among the tested compounds, the indanone-derived Schiff base showed the highest inhibition against *C. albicans*, while the indanone-chalcone and indanone-pyrazoline derivatives showed slightly lower inhibition.

Antimicrobial reference substances produced larger inhibition zones than synthesized substances. Tetracycline had inhibition zones of 32 and 34 mm against *E. coli* and *S. aureus*, respectively, while antifungal reference substances displayed more activity against the fungi that were tested. These data suggest that the synthesized compounds have some preliminary activity, although not as much as their corresponding reference substances.

Since just one synthesized indanone-pyrazoline compound was used, no structure-activity relationships could be developed based on these results. Additional studies with a greater number of structurally substituted indanone-pyrazoline compounds will be needed for such analysis.

4. Conclusions

In this study, a hybrid molecule of indanone and pyrazoline was synthesized using a three-step synthetic method, including Schiff base formation, chalcone formation, and cyclization of the chalcone with phenylhydrazine. The synthesized compounds were evaluated by ^1H NMR spectroscopy, with additional ^{13}C NMR data obtained for compounds 1 and 3. In the preliminary screening of antimicrobial activity, this hybrid molecule showed activity against *E. coli* and *S. aureus* but not against *A. flavus*. For the microorganism *C. albicans*, the indanone-pyrazoline hybrid molecule showed an inhibition zone of 10

mm, while the Schiff base molecule showed an 11 mm inhibition zone. Hence, this hybrid molecule does not show better activity against *C. albicans* than the Schiff base molecule. Further studies on a series of indanone-pyrazoline hybrids with different substituents, along with antimicrobial assays, are needed to establish the structure-activity relationship.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest. Ethical approval: All ethical guidelines have been adhered. Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Rabia Nazir, Muhammad Ifzan Arshad; Methodology: Rabia Nazir; Software: Rabia Nazir; Validation: Rabia Nazir; Formal Analysis: Rabia Nazir; Investigation: Rabia Nazir; Resources: Rabia Nazir; Data Curation: Rabia Nazir; Writing - Original Draft: Rabia Nazir, Muhammad Ifzan Arshad; Writing - Review and Editing: Rabia Nazir, Muhammad Ifzan Arshad; Visualization: Rabia Nazir; Funding acquisition: Muhammad Ifzan Arshad; Supervision: Muhammad Ifzan Arshad; Project Administration: Muhammad Ifzan Arshad.

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