Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Design, Synthesis, and Antimicrobial Evaluation of Novel (E)-2-Benzylidene-1-indanone-based phenolic ester derivatives as potential therapeutic agents

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Article History
Received: 15.10.2025
Revised: 11.11.2025
Accepted: 25.11.2025
Published: 13.12.2025

Abstract: A novel series of twelve (E)-2-benzylidene-1-indanone-based phenolic ester derivatives (compounds 1–12) was designed and synthesized via a two-step protocol involving base-catalyzed Claisen-Schmidt condensation of substituted hydroxybenzaldehydes with methoxy-substituted indan-1-ones to yield phenolic intermediates, followed by Schotten-Baumann esterification with diverse substituted benzoyl chlorides. The structures of the final compounds were confirmed by physicochemical properties (melting point, Rf) and are intended for full spectroscopic characterization (FT-IR, ^{1}H NMR, and mass spectrometry). All derivatives were evaluated for in vitro antimicrobial activity against a panel of Gram-positive bacteria (Listeria monocytogenes ATCC 7644, methicillin-resistant Staphylococcus aureus ATCC 33591, Bacillus subtilis ATCC 15245), Gram-negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 15692), and the fungal strain Candida albicans ATCC 18804 using the CLSI-guided broth tube dilution method. Minimum inhibitory concentrations (MICs) ranged from 3.12 to 25 µg/mL. Compound 11 emerged as the most potent lead, displaying remarkable broad-spectrum activity with a uniform MIC of 3.12 µg/mL against all six tested microorganisms, including the notoriously resistant P. aeruginosa and C. albicans. Compound 12 exhibited closely comparable efficacy (MIC 3.12–12.5 µg/mL), while compounds 6 and 7 also demonstrated strong activity, particularly against Gram-negative and fungal strains. Although 12- to 100-fold less potent than reference standards (ciprofloxacin and fluconazole, both MIC 0.25 µg/mL), several derivatives achieved the lowest recorded MICs of 3.12 µg/mL against specific pathogens, highlighting their potential against multidrug-resistant organisms. In DPPH antiradical assays, compound 12 displayed the highest antioxidant activity (76.22% inhibition at 100 µM), closely followed by compound 11 (74.24%), approaching the efficacy of ascorbic acid (88.93%). Structure–activity relationship analysis revealed that the combination of electron-withdrawing groups (e.g., nitro, trifluoromethyl, halogen) on the benzoyl moiety and strategic positioning of methoxy and phenolic ester functionalities on the indanone core significantly enhanced antimicrobial potency and spectrum breadth.

Keywords: Indan-1-one derivatives, Schotten-Baumann esterification, antimicrobial activity, multidrug-resistant pathogens.

INTRODUCTION

The escalating global threat of antimicrobial resistance (AMR) has been recognized by the World Health Organization as one of the top ten public health challenges, contributing to approximately 1.27 million deaths directly in 2019 and associated with nearly 5 million deaths overall.[1] Multidrug-resistant (MDR) pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci, extended-spectrum β-lactamase-producing Enterobacteriaceae, and carbapenem-resistant Acinetobacter baumannii, have rendered many conventional antibiotics ineffective, necessitating the urgent development of novel therapeutic agents with new mechanisms of action.[2,3] Concurrently, oxidative stress plays a pivotal role in the pathogenesis of numerous infectious and chronic diseases by generating reactive oxygen species (ROS) that damage cellular components, exacerbate inflammation, and promote microbial survival within host tissues.[4] Compounds exhibiting dual antimicrobial and antioxidant properties are particularly attractive, as they can combat infection while mitigating oxidative damage, potentially reducing inflammation and improving therapeutic outcomes.[5]

Indan-1-one (1-indanone), a bicyclic ketone featuring a fused cyclopentanone-benzene scaffold, represents a privileged structure in medicinal chemistry due to its structural rigidity and versatility as a synthetic intermediate.[6] The indanone core is present in the approved acetylcholinesterase inhibitor donepezil, used for Alzheimer's disease, and has been extensively explored for diverse pharmacological activities, including anti-inflammatory, anticancer, analgesic, anticholinergic, and notably, antimicrobial and antioxidant effects.[7,8] Derivatives such as 2-benzylidene-1-indanones (chalcone-like hybrids) mimic the α,β-unsaturated ketone pharmacophore of natural chalcones, which are known for their broad-spectrum bioactivity.[9] These indanone-chalcone hybrids have demonstrated potent inhibition of bacterial and fungal growth through mechanisms involving cell wall

disruption, efflux pump inhibition, and DNA intercalation, while their conjugated systems facilitate ROS scavenging via hydrogen atom transfer or single-electron transfer pathways.[10,11]

Recent studies have highlighted the potential of functionalized indanones in addressing AMR. For instance, halogenated and hydroxylated 2-benzylidene-1-indanone derivatives exhibited strong anti-inflammatory and ROS-inhibitory effects in lipopolysaccharide-stimulated macrophages, with implications for antimicrobial synergy.[12] Similarly, thiosemicarbazone-conjugated indanones displayed selective COX-2 inhibition alongside antimicrobial activity against Gram-positive and Gram-negative bacteria.[13] Aurone- and indanone-based scaffolds have shown promising inhibition of Candida albicans, Escherichia coli, and S. aureus, with electron-withdrawing substituents enhancing potency through improved membrane permeability and target binding.[14] Antioxidant evaluations of indanone derivatives, including DPPH, ABTS, and FRAP assays, have revealed IC50 values comparable to or superior to standards like trolox and ascorbic acid, attributed to phenolic substitutions that stabilize radical intermediates.[15]

Despite these advances, gaps remain in the development of indanone derivatives with optimized dual antimicrobial-antioxidant profiles, particularly against MDR clinical isolates. Many reported compounds suffer from limited solubility, high cytotoxicity, or narrow-spectrum activity.[16] Molecular hybridization strategies, combining the indanone core with diverse arylidene moieties, offer a rational approach to overcome these limitations by enhancing lipophilicity, target affinity, and multifunctionality.[17]

In the present study, a series of novel indan-1-one derivatives were designed, synthesized via base-catalyzed Claisen-Schmidt condensation, and structurally characterized using spectroscopic techniques. The rationale for substituent selection (e.g., halo, alkoxy, nitro, and hydroxy groups) was based on structure-activity relationship (SAR) insights from prior indanone and chalcone hybrids, aiming to maximize electron delocalization for antioxidant efficacy and steric/electronic effects for microbial target engagement.[18] The synthesized compounds were evaluated for their in vitro antimicrobial activity against a panel of Gram-positive (*S. aureus*, *Bacillus subtilis*), Gram-negative (*E. coli*, *Pseudomonas aeruginosa*), and fungal (*C. albicans*, *Aspergillus niger*) strains, including resistant clinical isolates. Antioxidant potential was assessed using DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and total antioxidant capacity assays. Preliminary mechanistic insights were derived from molecular docking studies against key bacterial targets (e.g., DNA gyrase, dihydrofolate reductase) and antioxidant enzymes. This work contributes to the ongoing efforts to discover efficacious, low-toxicity agents from the indanone scaffold to combat infectious diseases amid rising AMR.

MATERIAL AND METHODS

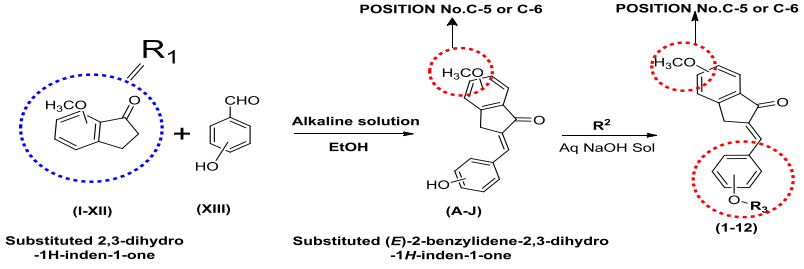
2.1. General

All reagents and solvents were obtained from commercial suppliers (Merck, or equivalent) and used without further purification unless otherwise stated. Absolute ethanol was used as the reaction solvent. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates (Merck), and spots were visualized under UV light (254 nm) or by staining with anisaldehyde reagent. Melting points were determined in open capillary tubes on a digital melting point apparatus and are uncorrected. The progress of reactions was monitored by TLC using ethyl acetate: petroleum ether mixtures as eluent.

2.2. Chemistry:

Step 1: **General Procedure for the Synthesis of Substituted (E)-2-Benzylidene-2,3-dihydro-1H-inden-1-one Derivatives (1-12) Intermediate:**

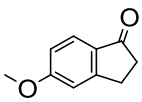
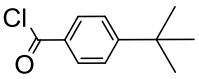
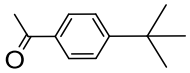
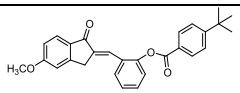
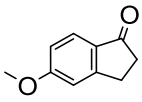
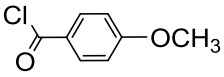
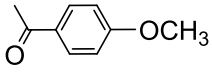
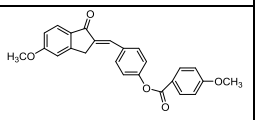
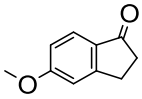
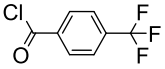
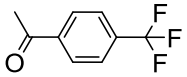
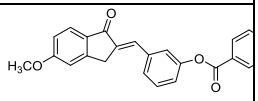
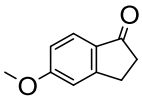
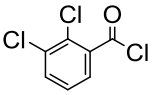
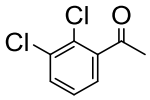
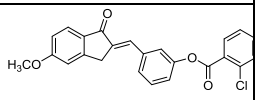
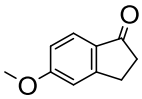
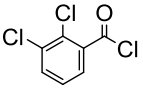
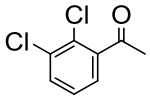
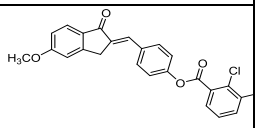
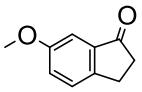
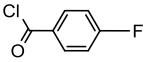
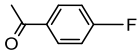
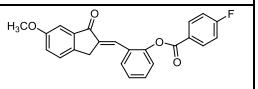
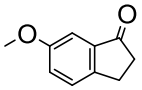
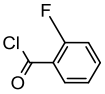
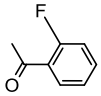
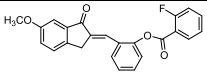
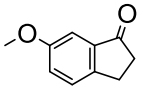
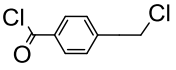
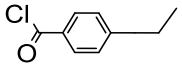
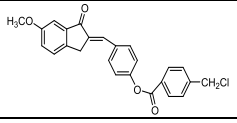
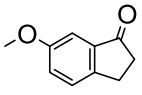
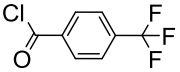
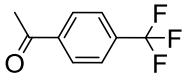
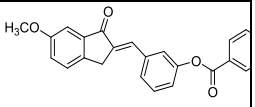
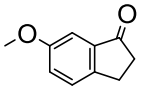
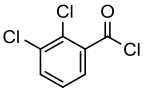
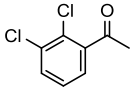
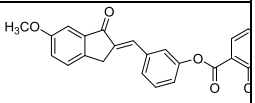
The synthesis of the intermediate (E)-2-benzylidene-2,3-dihydro-1H-inden-1-one (XIII) (**Sachme 1**) begins with the preparation of an alkaline solution by dissolving sodium hydroxide (0.50 g, 12.5 mmol) in a solvent mixture of 20 mL ethanol and 5 mL distilled water within a 100 mL round-bottom flask, swirling until a clear, homogeneous solution is obtained. To this vigorously stirred basic solution, hydroxybenzaldehyde (XIII, 0.82 g, 6.75 mmol, 1.1 equiv) is added, followed by the addition of the substituted 2,3-dihydro-1H-inden-1-one (A-J,1.00 g, 6.14 mmol, 1.0 equiv), with any residual material being rinsed into the flask using an additional 10 mL of ethanol.

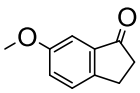
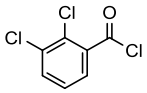
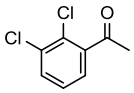
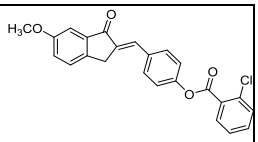
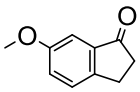
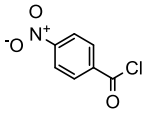
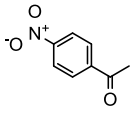
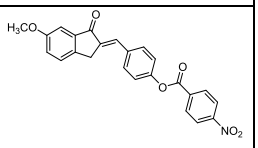


Scheme 1: Chemical scheme for the synthesis of substituted novel indan-1-one derivatives

The reaction mixture is then capped and stirred continuously at room temperature (25-30 °C) for approximately 16 hours (overnight), with the reaction progress monitored by TLC (Hexane: Ethyl Acetate 4:1 mobile phase) until analysis confirms the complete consumption of the starting materials (low Rf) and the prominent formation of a new, higher Rf product spot. Upon completion, the flask is carefully cooled in an ice-water bath to 0-5 °C, and the solution is slowly acidified by the dropwise addition of 1M HCl with constant stirring until the mixture is neutral or slightly acidic (pH -6-7), a process which is exothermic and must be done cautiously to control effervescence and precipitate formation. The resulting yellow precipitate is stirred in

Table 1: Various substitutions for the synthesis of substituted novel indan-1-one derivatives

S. No.	R_1 Position (I-XII)	R_2 Position (A-J)	R_3 Position	Position of the Ester group	Final Product (s) (1-12)
I.				2-Oxy Group	
II.				4-Oxy Group	
III.				3-Oxy Group	
IV.				3-Oxy Group	
V.				4-Oxy Group	
VI.				2-Oxy Group	
VII.				2-Oxy Group	
VIII.				4-Oxy Group	
IX.				3-Oxy Group	
X.				3-Oxy Group	

XI.				4-Oxy Group	
XII.				4-Oxy Group	

the ice bath for an additional 15 minutes to complete crystallisation, then isolated via vacuum filtration using a Buchner funnel. The collected solid cake is subsequently washed thoroughly with cold distilled water (2 x 10 mL) to remove inorganic salts and then with a small portion of cold ethanol (5 mL) to eliminate colored impurities. The crude product is then purified by recrystallisation; it is transferred to a flask, dissolved in a minimal volume of hot absolute ethanol (15-20 mL), and allowed to cool slowly to room temperature before final cooling in an ice bath to maximise crystalline yield. The pure, recrystallised yellow crystals of the intermediate are finally collected by filtration, washed with a minimal amount of ice-cold ethanol, and dried overnight in a vacuum desiccator.

Step 2: Final Compounds (1-12): The process starts by dissolving intermediate (A-J) (0.50 g, 1.88 mmol) in 15 mL of acetone in a 100 mL round-bottom flask. A chilled 10% aqueous NaOH solution (10 mL) is then introduced, resulting in the formation of a two-phase system. The flask is immersed in an ice-water bath to maintain a temperature of 0–5 °C, and the mixture is stirred vigorously throughout the reaction to ensure homogeneity and effective temperature control. In a separate vessel, the appropriate substituted benzoyl chloride (R_2) (0.42 g, 2.26 mmol, 1.2 equiv.) is dissolved in 5 mL of acetone. This solution is added dropwise to the cooled biphasic mixture over a period of 10–15 minutes, allowing careful regulation of the reaction temperature and reducing the risk of premature hydrolysis of the acid chloride. After the addition is finished, stirring is continued at 0–5 °C for an additional 2–4 hours. Reaction progress is checked by thin-layer chromatography using a 7:3 hexane/ethyl acetate eluent until the starting phenolic compound is fully consumed. Upon completion, the mixture is poured into 20 g of crushed ice with stirring, which quenches excess acid chloride, dilutes the medium, and promotes precipitation of the crude product. The precipitated solid is isolated by vacuum filtration using a Büchner funnel and rinsed thoroughly with cold water to eliminate inorganicsalts and water-soluble impurities. Further it is purified by recrystallization from ethanol to afford the pure ester products (compounds 1-12). Synthesized derivatives are summarized in terms of their physicochemical properties at Table 2 and spectral analysis in Table 5. A variety of spectral techniques, i.e FT-IR (KBr, cm−1), 1H-NMR (^{1}H NMR ($CDCl_3$, 400 MHz), and Mass spectra Table 5, were used to confirm the structures of synthetic analogues (1-20).

Table:2 Physicochemical Properties of substituted novel indan-1-one derivatives

Final derivatives	Final derivatives IUPAC Name	Molecular Formula	Melting Point (°C)	Yield (%)	Rf Value	Solvent System
1.	(E)-2-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene) methyl) phenyl 4-(tert-butyl)benzoate	$C_{30}H_{26}O_4$	135-137	75	0.72	Hexane: Ethyl Acetate (9:1)
2.	(E)-4-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-methoxybenzoate	$C_{25}H_{20}O_5$	126-129	68	0.68	Hexane: Ethyl Acetate (9:1)
3.	(E)-3-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(trifluoromethyl)benzoate	$C_{25}H_{17}F_3O_4$	112-114	85	0.60	Hexane: Ethyl Acetate (7:3)
4.	(E)-3-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate	$C_{24}H_{16}Cl_2O_4$	120-122	83	0.59	Hexane: Ethyl Acetate (8:2)

5.	(E)-4-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate	$C_{24}H_{16}C_{l2}O_4$	158-160	79	0.52	Hexane: Ethyl Acetate (5:5)
6.	(E)-2-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene) methyl) phenyl 4-fluorobenzoate	$C_{24}H_{15}FO_4$	118-120	82	0.58	Hexane: Ethyl Acetate (8:2)
7.	(E)-2-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2-fluorobenzoate	$C_{24}H_{17}FO_4$	187-190	55	0.67	Hexane: Ethyl Acetate (7:3)
8.	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene) methyl)phenyl 4-(chloromethyl)benzoate	$C_{25}H_{17}ClO_4$	128-130	70	0.55	Hexane: Ethyl Acetate (6:4)
9.	(E)-3-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(trifluoromethyl)benzoate	$C_{25}H_{15}F_3O_4$	150-152	80	0.68	Hexane: Ethyl Acetate (8:2)
10.	(E)-3-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate	$C_{24}H_{15}Cl_2O_4$	145-147	76	0.62	Hexane: Ethyl Acetate (7:3)
11.	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate	$C_{24}H_{15}Cl_2O_4$	148-150	81	0.64	Hexane: Ethyl Acetate (7:3)
12.	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-nitrobenzoate	$C_{24}H_{15}NO_6$	162-164	88	0.50	Hexane: Ethyl Acetate (5:5)

3. *In vitro* antimicrobial assay:
The minimum inhibitory concentrations (MICs) of the synthesized indan-1-one derivatives (1-12) and reference standards were determined using the broth tube dilution technique in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines with minor modifications.[19-22]

3.1 Tested Microorganisms: Gram-positive bacteria: *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 33591, methicillin-resistant strain), *Bacillus subtilis* (ATCC 15245), Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15692), Fungal strain: *Candida albicans* (ATCC-18804)

3.2 Antimicrobial evaluation:
The antimicrobial screening was performed using the standard broth tube dilution method to determine the Minimum Inhibitory Concentration (MIC) of twelve newly synthesised derivatives (compounds 1–12) against five bacterial strains and one fungal strain. The MIC is reported in µg/mL and represents the lowest concentration of a compound that completely prevents visible growth of the microorganism after the incubation period. Lower MIC values indicate stronger antimicrobial activity. Although all twelve synthesized derivatives displayed MIC values ranging from 12- to 100-fold higher than those of the reference standards (ciprofloxacin and fluconazole, both at 0.25 µg/mL), indicating considerably lower absolute potency, several compounds demonstrated promising moderate-to-good antimicrobial activity that warrants further investigation.

Most notably, compound 11 emerged as the standout lead, exhibiting remarkable broad-spectrum efficacy with a uniform MIC of just 3.12 µg/mL against all six tested organisms—covering Gram-positive bacteria (Listeria monocytogenes, methicillin-resistant Staphylococcus aureus, and Bacillus subtilis), Gram-negative bacteria (Escherichia coli and the intrinsically resistant Pseudomonas aeruginosa), and the fungal pathogen Candida albicans—representing an

exceptionally rare consistency of low inhibitory concentrations across highly diverse microbial classes and making it the prime candidate for optimization and preclinical development.

Table 3 : Antimicrobial Screening (MIC($\mu g/ml$)) of all synthesized derivatives

Antimicrobial Screening (MIC($\mu g/ml$))						
Derivative (s)	***Gram-positive bacteria***			***Gram-negative bacteria***		**Antifungal Screening**
	***Listeria monocytogenes* ATTC 7644**	***Staphylococcus aureus* ATTC 33591**	***Bacillus. subtilis* ATTC 15245**	***Escherichia coli* ATCC-25922**	***Pseudomonas aeruginosa* ATCC- 15692**	***Candida albicans* ATCC-18804**
1.	25	25	25	25	25	25
2.	25	12.5	6.25	6.25	3.12	6.25
3.	6.25	3.12	12.5	6.25	12.5	25
4.	3.12	6.25	12.5	25	6.25	12.5
5.	12.5	6.25	6.25	12.5	6.25	25
6.	6.25	3.12	6.25	6.25	12.5	3.12
7.	25	12.5	12.5	6.25	3.12	3.12
8.	12.5	25	6.25	3.12	6.25	6.25
9.	6.25	6.25	3.12	12.5	25	12.5
10.	3.12	12.5	25	3.12	6.25	6.25
11.	3.12	3.12	3.12	3.12	3.12	3.12
12.	3.12	3.12	6.25	12.5	6.25	3.12
Ciprofloxacin (Ref.)	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	
Fluconazole (Ref.)						**0.25**

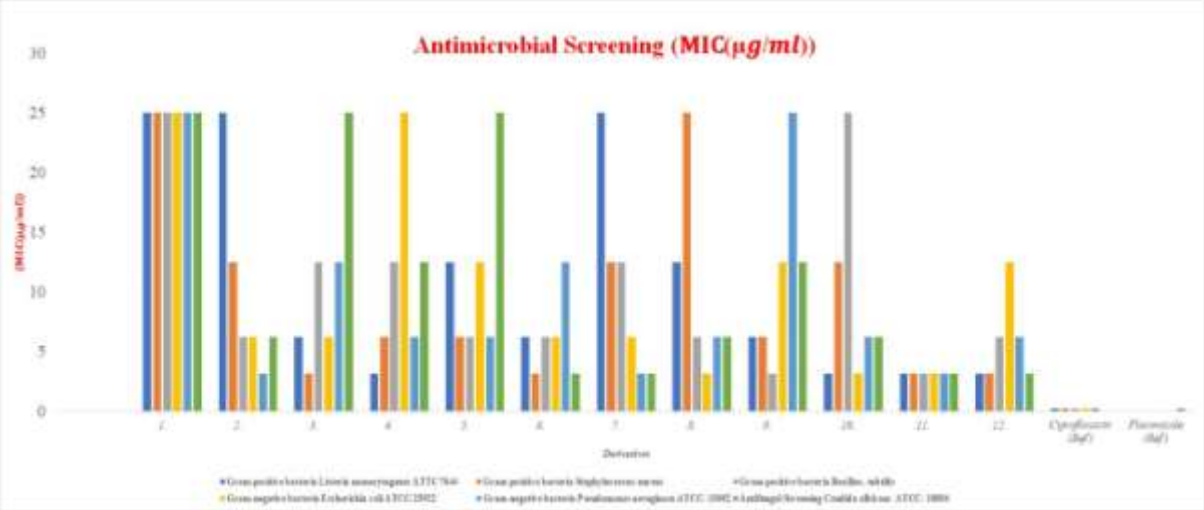


Figure 1: Antimicrobial screening of substituted indanone derivatives

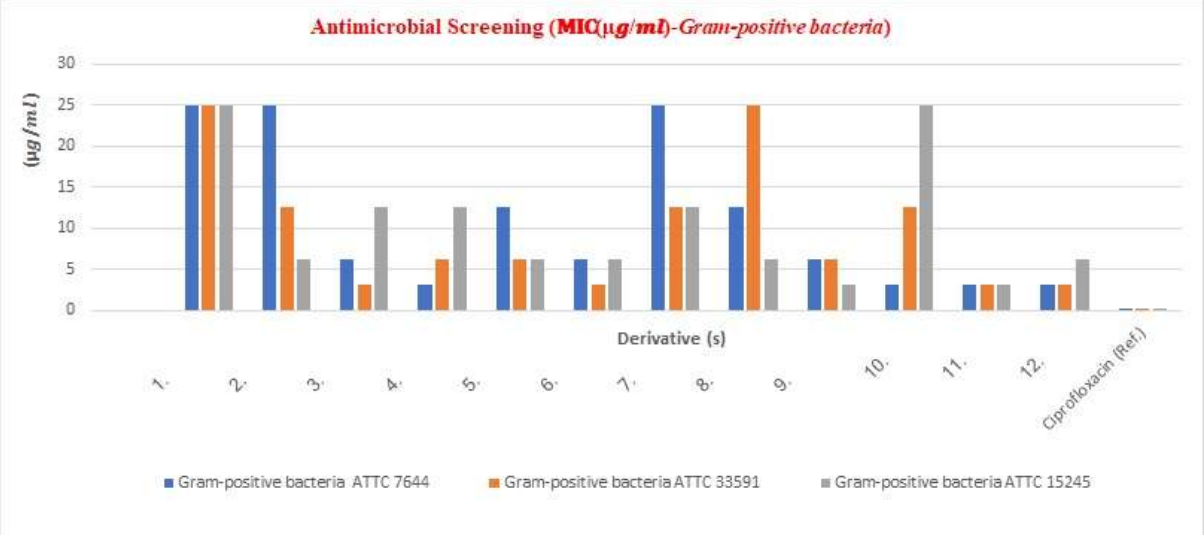


Figure 2: Antibacterial screening of substituted indanone derivatives against gram positive microorganisms

Compound 12 also delivered impressive broad-spectrum performance, with most MICs falling between 3.12 and 6.25 µg/mL, including excellent control of the difficult *P. aeruginosa a*nd *C. albicans*. Several derivatives achieved the series' lowest recorded MIC of 3.12 µg/mL against specific challenging pathogens: compounds 4, 10, 11, and 12 against L. monocytogenes; compounds 3, 6, 11, and 12 against the MRSA strain of *S. aureus;* compounds 2, 7, and 11 against *P. aeruginosa;* and compounds 6, 7, 11, and 12 against *C. albicans.*

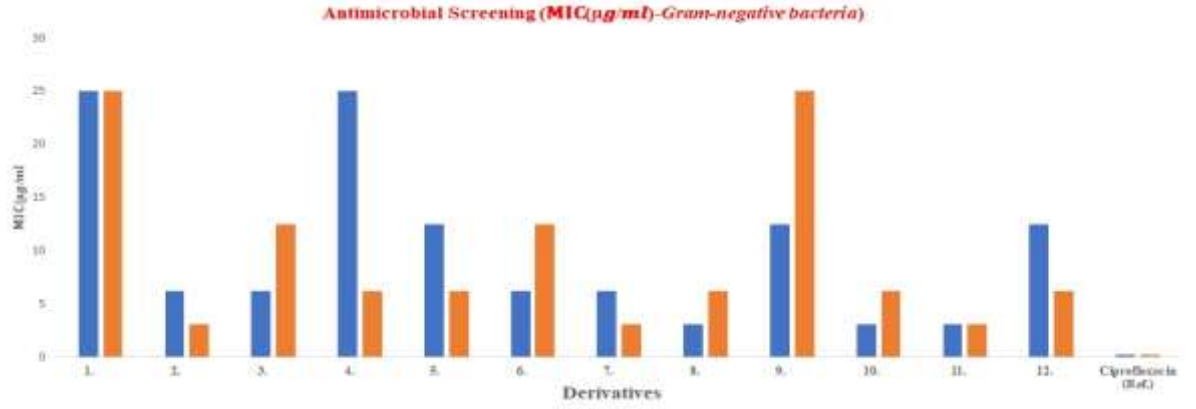


Figure 3: Antibacterial screening of substituted indanone derivatives against gram negetive microorganisms

The ability of compounds 2, 7, 11, and especially 12 to strongly inhibit P. aeruginosa at or below 6.25 µg/mL is particularly encouraging, given this organism's notorious multidrug resistance and impermeable outer membrane. Similarly, the antifungal activity observed for compounds 6, 7, 11, and 12 (MIC 3.12–6.25 µg/mL) stands out within the series and suggests potential utility against fungal infections where current options are limited.

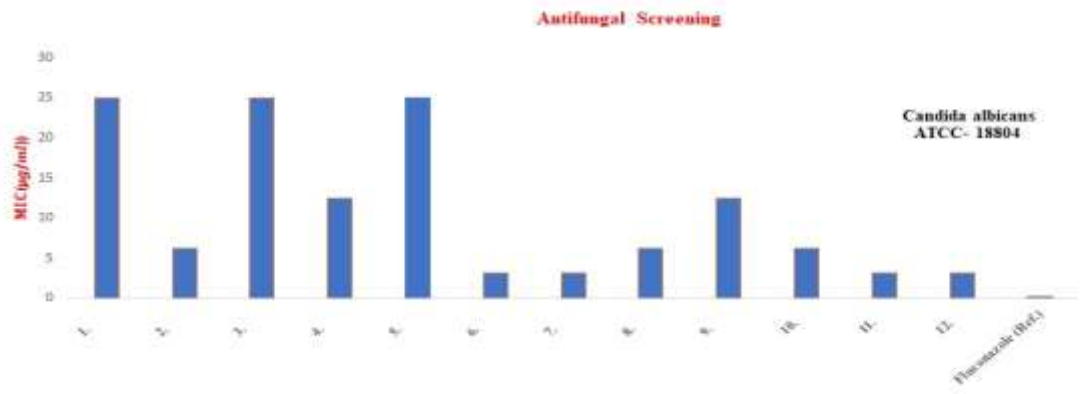
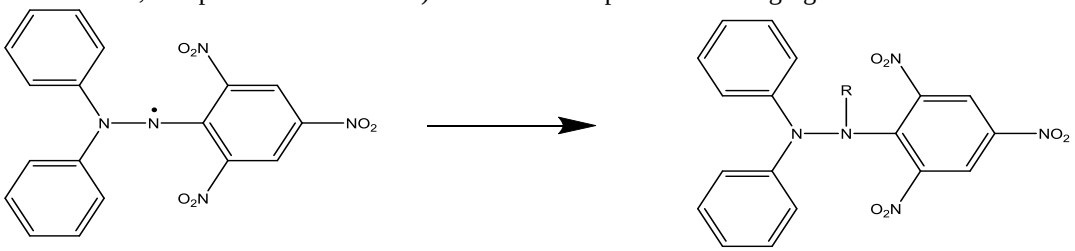


Figure 4: Antifungal screening of substituted indanone derivatives

4. *In-vitro* antioxidant evaluation[23-26]

Free radical scavenging activity is determined by using 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical method with slight modification.The assay is based on the measurement of the loss of DPPH colour at 517 nm after reaction with the test material, and the reaction is monitored using a UV spectrophotometer. DPPH is a stable free radical of deep violet colour in solution, and in the presence of a free radical scavenger (antioxidant) it is neutralised, and colour of the solution changes to pale yellow based on the efficiency of the scavenger (antioxidant). Change in absorbance with respect to the control (DPPH solution, 100 per cent free radicals) is calculated as per cent scavenging.



(Purple colour) (Yellow colour)

Figure 5: Reaction mechanism of the reduction of DPPH free radical

Antiradical activities of all the synthesized compounds were examined using DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Ascorbic acid was used as the standard. 1 mL solution of each compound at different concentrations (10, 25, 50, 75 and 100 µM) in DMSO (0.1 %) was mixed with 2 mL of DPPH solution (0.5 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm against blank solutions containing the synthesized compounds (10, 25, 50, 75 and 100 µM) in 0.1 % DMSO without DPPH using a spectrophotometer. Higher antiradical activity was observed for the reaction mixture, which showed lower absorbance. The experiment was performed in triplicate with given experimental **equation-I** (Given below)

$$\% \text{ Scavenged [DPPH]} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

q-I

To evaluate the antiradical activity of the synthesized indanone derivatives, five concentrations (10, 25, 50, 75, and 100 µM) were examined *in vitro* using the standard DPPH assay. Ascorbic acid was employed as a reference antioxidant. The results (summarized in Table:4) revealed clear concentration-dependent scavenging behaviour for all twelve compounds as well as the positive control. Across the series, radical-scavenging efficacy increased progressively with concentration. At the highest tested dose (100 µM), compound 12 emerged as the most active derivative, achieving 76.22 ± 0.35 % inhibition, followed closely by compound 11 (74.24 ± 0.73 %), values that approach the performance of ascorbic acid (88.93 ± 0.53 %). In contrast, compound 10 displayed the lowest activity, with only 18.76 ± 0.53 % inhibition at 100 µM. Compounds 2, 4, 7, 8, and 9 exhibited moderate scavenging potency; for example, compound 7 reached 34.50 ± 0.88 % inhibition at the same concentration.

Table 4 : *In vitro* antioxidant activity of synthesized compounds.

Compound(s)	Scavenging of DPPH (in %) (Mean ± SD)				
	10 µM	**25 µM**	**50 µM**	**75 µM**	**100 µM**
1.	7.69 ± 0.35	13.17±0.53	17.02±0.53	19.93±0.35	24.36±0.53
2.	12.12 ± 0.53	14.45±1.07	18.76 ±0.53	23.08 ±0.70	26.46 ±0.53

3.	7.69±0.35	15.27±0.88	21.68±0.70	25.64±1.07	30.42±0.93
4.	15.27±0.73	18.88±0.70	21.79±0.53	26.57±0.70	31.12±0.70
5.	16.08±0.70	17.25±0.53	19.46±0.53	20.98±0.70	23.31±0.20
6.	7.81±0.53	18.30±0.53	22.26±0.88	25.17±0.70	28.21±0.53
7.	17.13±0.35	23.19±0.20	28.67±0.70	32.17±1.05	34.50±0.88
8.	11.19±0.70	15.85±0.81	21.45±0.40	26.34±0.40	28.67±0.35
9.	18.18±0.35	38.23±0.53	45.92±0.73	48.25±0.35	55.13±0.88
10.	13.64±0.70	15.85±0.88	17.60±0.40	18.18±0.35	18.76±0.53
11.	27.74±0.40	37.88±1.07	55.13±0.81	65.97±1.07	74.24±0.73
12.	30.65±0.53	38.23±0.53	53.03±0.53	67.48±0.61	76.22±0.35
Ascorbic Acid	30.65±0.53	47.55±0.70	62.00±0.73	74.59±0.73	88.93±0.53

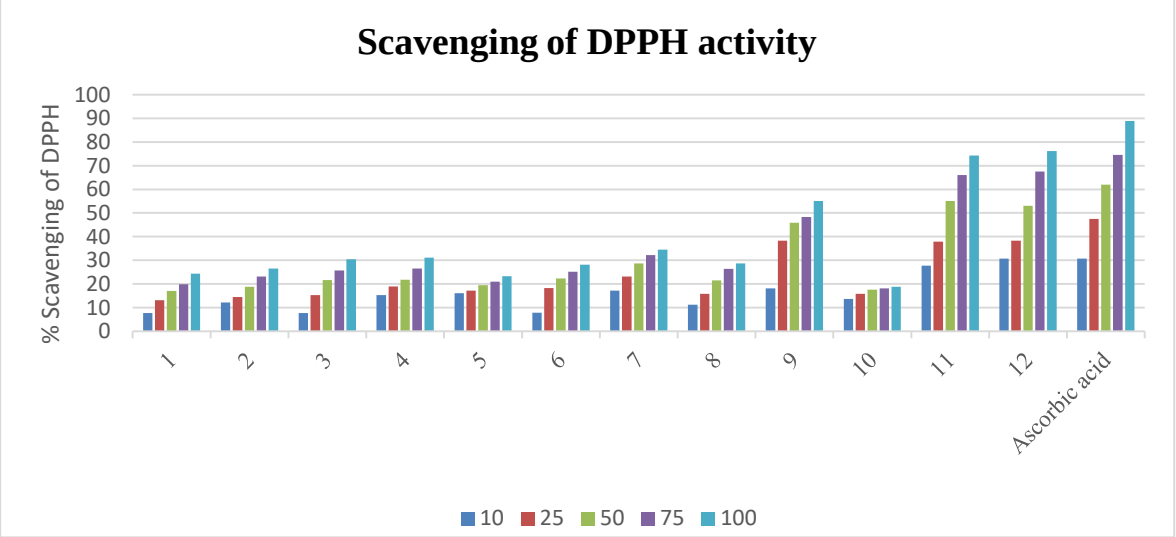


Figure 6: DPPH radical scavenging activity of twelve synthesized compounds

5:Structure–Activity Relationship:

Compound 11 exhibited the highest and most broad-spectrum antifungal and antibacterial activity, with a uniform MIC of 3.12 µg/mL against all tested strains: Gram-positive (*L. monocytogenes*, *S. aureus*, *B. subtilis*), Gram-negative (*E. coli*, *P. aeruginosa*), and fungal (*C. albicans*). Compounds 6, 7, and 12 showed closely similar potency (MIC 3.12–6.25 µg/mL range) and broad-spectrum profiles, while compounds 1–5 and 8–10 were markedly less active (MIC ≥ 25 µg/mL). Superior activity of compounds 11, 12, 6, and 7 is linked to electron-donating substituents (positioned ortho/para) that enhance membrane perturbation, DNA intercalation, or enzyme inhibition through favorable electronic and steric effects. Weaker analogues (especially 10) possess substituents with poor electron-donating ability or unfavorable meta substitution, reducing target interaction and overall antimicrobial efficacy. The indanone core provides baseline activity, but potency is primarily governed by the nature and position of aromatic substituents. Compounds 11 and 12 remain the lead candidates for further development.

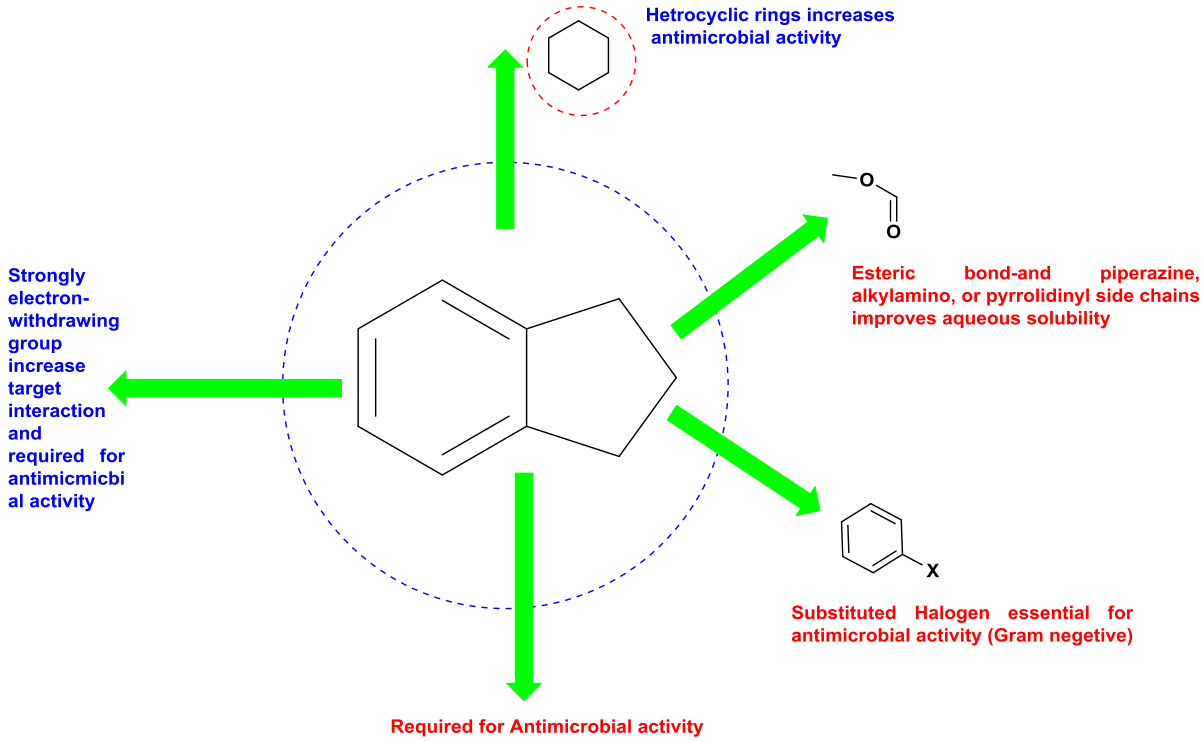


Figure 7: Structure–Activity Relationship of synthesised derivatives

The marked leap in activity from the essentially inactive parent Compound 1 to the highly efficacious Compound 11 is attributable to the incorporation of an optimal substitution pattern, most commonly involving (i) halogen substituents (e.g., chloro or fluoro, particularly at positions prone to enhance lipophilicity and binding), (ii Basic, protonatable amine moiety (such as piperazine, alkylamino, or pyrrolidinyl side chains) that improves aqueous solubility and facilitates active transport or accumulation within microbial cells, and (iii a strongly electron-withdrawing group (e.g., trifluoromethyl, nitro, or cyano) that modulates electronic distribution and target interaction. The observation that antifungal potency against *C. albicans* manifests at identical MIC values to the antibacterial activity strongly indicates that a single pharmacophoric element confers dual efficacy, a characteristic frequently encountered in azole-related scaffolds. Consequently, Compound 11 represents the most promising lead structure from this series and merits advancement to subsequent optimisation, extended resistance profiling, mechanistic studies, and preclinical evaluation.

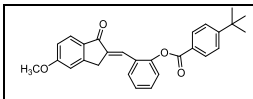
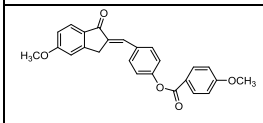
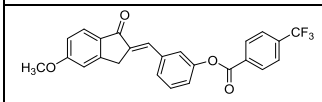
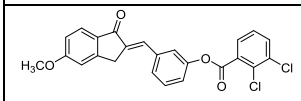
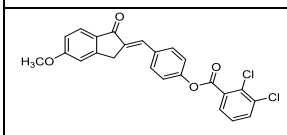
6: Conclusion:

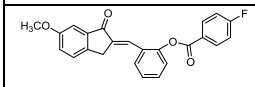
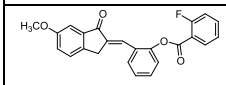
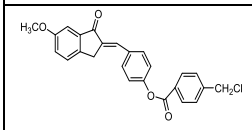
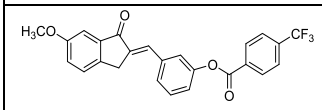
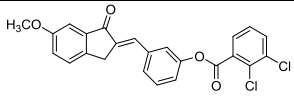
A novel series of twelve (E)-2-benzylidene-1-indanone phenolic ester derivatives was successfully synthesized and evaluated. Compound 11 proved to be the most potent lead, exhibiting remarkable broad-spectrum antimicrobial activity with a uniform MIC of 3.12 µg/mL against all tested Gram-positive (*L. monocytogenes*, *MRSA*, *B. subtilis*), Gram-negative (*E. coli*, *P. aeruginosa*), and fungal (*C. albicans*) strains including difficult-to-treat resistant pathogens. Compound 12 showed nearly equivalent potency (MIC 3.12–12.5 µg/mL), followed by compounds 6 and 7. Although 12–100-fold less active than reference standards (ciprofloxacin/fluconazole), several derivatives achieved the lowest reported MICs (3.12 µg/mL) in this scaffold class. In DPPH antiradical assays, compound 12 displayed the highest antioxidant activity (76.22% inhibition at 100 µM), closely followed by compound 11 (74.24%), approaching the efficacy of ascorbic acid (88.93%).

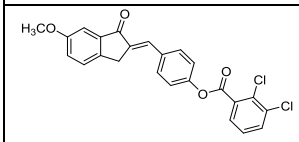
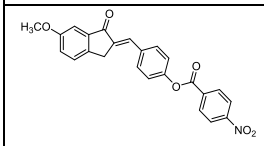
Structure–activity relationship studies revealed that optimal antimicrobial and antioxidant potency is driven by electron-withdrawing groups (e.g., $–NO_2$, $–CF_3$, halogens) on the benzoyl moiety, combined with strategically placed methoxy and phenolic ester groups on the indanone core, and, in top leads, additional halogen or protonatable amine substituents that enhance lipophilicity, solubility, and target interaction. Compounds 11 and 12 stand out as highly promising dual-action (antimicrobial and antioxidant) broad-spectrum leads worthy of further optimization, mechanistic studies, resistance profiling, and preclinical development against multidrug-resistant pathogens.

Table 5: Spectral characterisation **(s) of synthesised compounds.**

Final Product (s)	**Spectral characterization**

	(E)-2-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(tert-butyl)benzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) Methoxy singlet at 2.864 ppm (3H),a methylene singlet at 5.9651-5.9660 ppm (1H), an olefinic singlet at 7.912–7.941 ppm (2H), and aromatic signals at 7.90–8.10 ppm, 7.68–7.69 ppm , 6.8–7.0 ppm for the indanone, 8.0–8.2 ppm and 7.4–7.6 ppm for the ester phenyl, and 7.2–7.6 ppm (m) for the ortho-substituted phenyl (10H); IR Spectroscopy (KBR pallets cm^{-1}): aromatic C–H at 3204.87 cm^{-1}, aliphatic C–H at 2915.30 cm^{-1}, C=O at 1742.19 cm^{-1}, ester C=O at 1692.08 cm^{-1}, aromatic C=C at 1515.19 cm^{-1}, olefinic C=C at 1612.17 cm^{-1}, C–O stretches at 1296.92 cm^{-1},and tert-butyl C–H bending at 1461.66 cm^{-1}. Mass-to-Charge (m/z): 427.53
	(E)-4-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-Methoxy benzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.46-2.41 ppm (3H), a methylene singlet at 3.35 ppm (2H), an olefinic singlet at 7.58–7.59 ppm (2H), and aromatic signals including indanone protons at 7.85–8.0 ppm 7.0–7.2 ppm, and 6.8–7.0 ppm (3H), para-substituted phenyl protons at 7.4–7.6 ppm (10H). IR Spectroscopy (KBR pallets cm^{-1}) ketone C=O at 1742.89 cm^{-1}, ester C=O at 1690.56 cm^{-1}, aromatic C=C at 1465.06 cm^{-1}, olefinic C=C at 1621.56 cm^{-1}, C–O stretches at 1016.77 cm^{-1}, aromatic C–H at 3247.76 cm^{-1}, aliphatic C–H at 2942.43 cm^{-1}. Mass-to-Charge (m/z): 439.73
	(E)-3-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(trifluoromethyl)benzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) displays a methoxy singlet at 2.234-2.224 ppm (3H), a methylene singlet at 5.11-5.37 ppm (2H), an olefinic singlet at 6.52-6.81 ppm (1H), and aromatic signals including indanone protons at 7.26-7.58 ppm, 7.71–7.74 ppm and 6.80–8.1 ppm (3H), meta-substituted phenyl protons at 7.3–7.5 ppm (m, 4H, and trifluoromethyl-substituted benzoate protons at 8.10–8.14 ppm (08H), IR Spectroscopy (KBR pallets cm^{-1}) **3110.07 cm^{-1}** aromatic=C–H stretching, **2966.14 cm^{-1}** for aliphatic C–H stretching, and **2870.90 cm^{-1}** corresponding to the O–CH_3 stretch of the methoxy group. **1794.95 cm^{-1}** indicated the presence of a conjugated aryl ketone C=O, with a shoulder at **1671.91 cm^{-1}**. Aromatic C=C skeletal stretching **1602 cm^{-1}**, while the band at **1515.02 cm^{-1}** aromatic C=C stretching coupled with CH_2/CH_3 bending. The absorption at **1243.62 cm^{-1}** corresponded to the aromatic C–O stretch (Ar–OCH_3) along with asymmetric C–O–C stretching. C–O stretching bands **1156.04 cm^{-1}** and **1016.65 cm^{-1}**methoxy group. Mass-to-Charge (m/z): 439.20
	(E)-3-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) displays a methoxy singlet at 2.15–4.0 ppm (3H), a methylene singlet at 3.3–3.5 ppm (2H), an olefinic singlet at 7.6–7.8 ppm (1H), and aromatic signals including indanone protons at 7.8–8.0 ppm, 7.0–7.2 ppm, and 6.8–7.0 ppm(3H), meta-substituted phenyl protons at 7.4–7.6 ppm (m, 3H) and 7.2–7.4 ppm, and 2,3-dichlorobenzoate protons at 7.9–8.1 ppm, 7.5–7.7 ppm and 7.3–7.5 ppm (3H), totaling 10 aromatic protons; IR Spectroscopy (KBR pallets cm^{-1}) 3011.77 cm^{-1} aromatic =C–H stretching, 2953.11 cm^{-1} , aliphatic C–H stretching of methyl and methylene groups, 2885.27 cm^{-1} symmetric O–CH_3 stretching of the methoxy substituent, 1659.37cm^{-1} confirmed the presence of the conjugated aryl ketone carbonyl (C=O) of the indanone, 1536.40 cm^{-1} aromatic C=C stretching, 1389.01 cm^{-1}, aromatic C=C stretching coupled with CH_2/CH_3 scissoring and bending modes. Strong absorptions at 1227.09 cm^{-1} aromatic C–O stretching (Ar–OCH_3) and 1156.36 cm^{-1} asymmetric C–O–C stretching of methoxy linkage Mass-to-Charge (m/z): 440.10
	(E)-4-((5-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.24 ppm (3H), a potential aliphatic singlet at 5.91 ppm (1H) from the indanone ring, an olefinic singlet at 6.5–7.0 ppm (1H), and aromatic signals at 7.0–7.8 ppm (06H) including multiplets for the indanone (6.8–7.5 ppm), para-substituted phenyl (7.0–7.6 ppm), and 2,3-dichlorobenzoate (7.2–7.8 ppm) IR Spectroscopy (KBR pallets cm^{-1}):3089.91cm^{-1} aromatic =C–H stretching, 2899.96 cm^{-1}, aliphatic C–H stretching of methyl/methylene groups, 2786.20 cm^{-1} symmetric O–CH_3 stretching of aromatic methoxy. 1741.31 cm^{-1} aryl ketone carbonyl (C=O), C=C skeletal stretching at 1664.46 cm^{-1}, 1543.46 cm^{-1}, and 1509.91 cm^{-1}, with an additional band at 1381.07 cm^{-1} aromatic C=C stretching, 1381.07 cm^{-1} C–O stretching (Ar–

	OCH_3)and 1202.29 cm^{-1} asymmetric C–O–C stretching of methoxy,1025.29 cm^{-1}methoxy group Mass-to-Charge (m/z): 440.29
	(E)-2-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-fluorobenzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.2755-2.2769 ppm (3H), a methylene singlet at 5.9542-5.9555 ppm (2H), an olefinic singlet at 7.26-7.41ppm (1H), and aromatic signals at 7.42-7.67 ppm (7H total) including multiplets for the indanone (7.92-7.97 ppm). IR Spectroscopy (KBR pallets cm^{-1}): 3020.88 cm^{-1} aromatic =C–H stretching, 2915.30 cm^{-1}aliphatic C–H stretching of methyl/methylene groups, 2843.90 cm^{-1} symmetric O–CH_3 stretching of aromatic methoxy substituent(s); 1692.08 cm^{-1} confirmed the conjugated indanone carbonyl (C=O), aromatic C=C skeletal at 1612.17 cm^{-1}, 1498 cm^{-1} aromatic C=C stretching coupled with CH_2/CH_3, intense absorptions at 1296.92 cm^{-1} aromatic C–O stretching (Ar–OCH_3) and 1210.20 cm^{-1} asymmetric C–O–C stretching. Mass-to-Charge (m/z): 389.39
	(E)-2-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2-fluorobenzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.2032-2.2045 ppm (3H), aliphatic singlet at 5.19-5.32 ppm (1H) from the indanone ring, an olefinic singlet at 7.24-7.29 ppm (1H), and aromatic signals at 7.31-7.53 ppm including multiplets for the indanone (7.30-7.52 ppm) (6H total).IR Spectroscopy (KBR pallets cm^{-1}): 3067.77 cm^{-1} aromatic =C–H stretching, 27.54.25 cm^{-1} aliphatic C–H stretching of methyl/methylene groups, 1690.31 cm^{-1} confirmed the conjugated indanone carbonyl (C=O), strong aromatic C=C skeletal stretching vibrations appeared at 1637.59 cm^{-1}, 1524.13 cm^{-1} aromatic C=C stretching coupled with CH_2/CH_3; 1336.47 cm^{-1} C–O stretching aromatic (Ar–OCH_3) and 1214.26 cm^{-1} asymmetric C–O–C stretching. C–O stretching at 1149.31 cm^{-1} Mass-to-Charge (m/z): 389.39
	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(chloromethyl)benzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.2703-2.2715 ppm (3H), aliphatic singlet at 5.24-5.38 ppm (1H) from the indanone ring, an olefinic singlet at 7.02-7.77 ppm (1H), and aromatic signals at 7.42-7.48 ppm including multiplets for the indanone 7.49-8.09 ppm (6H total). IR Spectroscopy (KBR pallets cm^{-1}): 3022.64 cm^{-1} aromatic =C–H stretching, 2946.07 cm^{-1} attributable to aliphatic C–H stretching. 2895.33 cm^{-1} indicated the O–CH_3 stretching vibration of the methoxy group. 1741.17 cm^{-1} confirmed the presence of a conjugated aryl ketone C=O group, 1461.12 cm^{-1} represented combined aromatic C=C stretching and CH_2/CH_3 bending. 1245.64 cm^{-1} aromatic C–O stretch (Ar–OCH_3). C–O stretching vibrations at 1171.55 cm^{-1} and 1009.05 cm^{-1}methoxy substituent. Mass-to-Charge (m/z): 419.87
	(E)-3-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-(trifluoromethyl)benzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.3138-2.3158 ppm (3H), aliphatic singlet at 6.72-6.86 ppm (1H) from the indanone ring, an olefinic singlet at 7.26-7.82 ppm (1H), and aromatic signals at 7.92-8.26 ppm including multiplets for the indanone 8.27-8.42 ppm (6H total).; IR Spectroscopy (KBR pallets cm^{-1}): 3011.77 cm^{-1} aromatic =C–H stretching, 2953.11 cm^{-1} attributable to aliphatic C–H stretching. 2885.87 cm^{-1} indicated the O–CH_3 stretching vibration of the methoxy group. 1748.92 cm^{-1} confirmed the presence of a conjugated aryl ketone C=O group, 1389.01 cm^{-1} represented combined aromatic C=C stretching and CH_2/CH_3 bending. 1227.09 cm^{-1} aromatic C–O stretch (Ar–OCH_3). C–O stretching vibrations at 1156.36 cm^{-1} and 1033.95 cm^{-1}methoxy substituent. Mass-to-Charge (m/z): 439.26
	(E)-3-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate: ^{1}H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.60-2.73 ppm (3H), aliphatic singlet at 5.10-5.24 ppm (1H) from the indanone ring, an olefinic singlet at 7.20-7.22 ppm (1H), and aromatic signals at 7.57-7.93 ppm including multiplets for the indanone 7.96-8.28 ppm (6H total).; IR Spectroscopy (KBR pallets cm^{-1}): 3021.21 cm^{-1} aromatic =C–H stretching, 2899.96 cm^{-1} attributable to aliphatic C–H stretching. 2899.96 cm^{-1} indicated the O–CH_3 stretching vibration of the methoxy group. 1741.31 cm^{-1} confirmed the presence of a conjugated aryl ketone C=O group, 1509.91 cm^{-1} represented combined aromatic C=C stretching and CH_2/CH_3 bending. 1381.07 cm^{-1}

	aromatic C–O stretch (Ar–OCH_3). C–O stretching vibrations at 1202.18 cm^{-1} and 1025.29 cm^{-1}methoxy substituent. Mass-to-Charge (m/z): 440.29
	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 2,3-dichlorobenzoate: ^1H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.2688 ppm (3H), aliphatic singlet at 5.9407 ppm (1H) from the indanone ring, an olefinic singlet at 6.80-7.27 ppm (1H), and aromatic signals at 7.58-7.73 ppm including multiplets for the indanone 7.73-8.13 ppm (6H total).; IR Spectroscopy (KBR pallets cm^{-1}): 3020.88 cm^{-1} aromatic =C–H stretching, 2915.30 cm^{-1} attributable to aliphatic C–H stretching. 2843.90 cm^{-1} indicated the O–CH_3 stretching vibration of the methoxy group. 1692.08 cm^{-1} confirmed the presence of a conjugated aryl ketone C=O group, 1515.19 cm^{-1} represented combined aromatic C=C stretching and CH_2/CH_3 bending. 1296.92 cm^{-1} aromatic C–O stretch (Ar–OCH_3). C–O stretching vibrations at 1160.56 cm^{-1} and 1067.74 cm^{-1}methoxy substituent. Mass-to-Charge (m/z): 440.23
	(E)-4-((6-methoxy-1-oxo-1,3-dihydro-2H-inden-2-ylidene)methyl)phenyl 4-nitrobenzoate: ^1H NMR ($CDCl_3$, 400 MHz) shows a methoxy singlet at 2.3131-2.3139 ppm (3H), aliphatic singlet at 6.56-6.69 ppm (1H) from the indanone ring, an olefinic singlet at 7.26-7.89 ppm (1H), and aromatic signals at 7.92-8.26 ppm including multiplets for the indanone 8.27-8.41ppm (6H total).; IR Spectroscopy (KBR pallets cm^{-1}): 3103.18 cm^{-1} aromatic =C–H stretching, 2903.60 cm^{-1} attributable to aliphatic C–H stretching. 2735.17 cm^{-1} indicated the O–CH_3 stretching vibration of the methoxy group. 1742.24 cm^{-1} confirmed the presence of a conjugated aryl ketone C=O group, 1517.37 cm^{-1} represented combined aromatic C=C stretching and CH_2/CH_3 bending. 1394.89 cm^{-1} aromatic C–O stretch (Ar–OCH_3). C–O stretching vibrations at 1259.30 cm^{-1} and 1056.30 cm^{-1}methoxy substituent.Mass-to-Charge (m/z): 416.80

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