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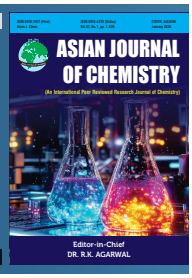

Novel Isatin-Chalcone Derivatives: Synthesis, Characterization and Evaluation for Antimicrobial and Anticancer Applications

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A series of novel isatin-chalcone hybrids (**IA-IM**) were synthesized *via* a microwave-assisted method and evaluated for their antimicrobial, antifungal, antitubercular and anticancer activities. The compounds were characterized using spectroscopic techniques and screened for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, antifungal activity against *Candida albicans* and *Aspergillus niger*, antitubercular activity against *Mycobacterium tuberculosis* H37Rv and cytotoxicity against breast (MCF-7) and cervical (HeLa) cancer cell lines. Compounds **IK** (4-NH_2), **IF** (2-Cl) and **IL** (2-F) exhibited potent antibacterial activity, with zones of inhibition (ZOI) comparable to or exceeding amikacin at higher concentrations. Against fungal strains, **IM** (4-NO_2), **IK** (4-NH_2) and **IL** (2-F) demonstrated significant activity, approaching the efficacy of fluconazole. In antitubercular assays, compounds **ID** (2-F), **IE** (3,4-OCH_3), **IF** (2-Cl) and **IG** (4-Cl) showed sensitivity at 12.5-25 µg/mL. Anticancer evaluations revealed that compounds **IH** (4-Br), **IK** (4-NH_2) and **IE** (3,4-OCH_3) were highly potent against MCF-7 and HeLa cells, with IC_{50} values as low as 6.53 ± 1.12 µM, while sparing normal HEK-293 cells. Structure-activity relationship (SAR) studies highlighted the importance of electron-donating groups (*e.g.* -NH_2, -OCH_3) and halogen substitutions (*e.g.* -Cl, -Br) in enhancing biological activity. These findings demonstrate the potential of isatin-chalcone hybrids as multi-functional therapeutic agents, providing a foundation for further optimization and development.

Keywords: Isatin, Chalcone, Antibacterial, Antifungal, Anti-tubercular, Anticancer.

INTRODUCTION

The emergence of multidrug-resistant (MDR) pathogens [1,2] and the increasing prevalence of life-threatening microbial infections, tuberculosis (TB) [3] and cancer [4,5] have underscored the urgent need for the development of novel therapeutic agents with broad-spectrum biological activities. Traditional approaches to drug discovery often fall short in addressing the complexity of these diseases, necessitating the exploration of innovative chemical scaffolds that can target multiple pathways simultaneously [6-8]. In this context, hybrid molecules, which combine two or more pharmacophores into a single entity, have gained significant attention due to their potential to enhance efficacy, reduce resistance and minimize side effects [9].

Isatin (1*H*-indole-2,3-dione), a privileged heterocyclic scaffold, has been extensively studied for its diverse pharmacological properties, including antimicrobial, antitubercular, antifungal and anticancer activities [10,11]. Its ability to interact with multiple biological targets makes it an attractive candidate for the design of hybrid molecules [12]. On the other hand, chalcones (1,3-diaryl-2-propen-1-ones) are another class of bioactive compounds known for their wide range of biological activities, including anti-inflammatory, antimicrobial and anticancer effects [13-15]. The α,β-unsaturated ketone moiety in chalcones is particularly significant, as it facilitates interactions with cellular targets through Michael addition reactions, making them versatile building blocks in medicinal chemistry [16,17].

Recent studies [18-20] have demonstrated that the conjugation of isatin with chalcone moieties can lead to synergistic effects, enhancing the biological potency of the resulting hybrids. For instance, isatin-chalcone hybrids have shown promising activity against drug-resistant bacterial strains, fungal pathogens and *Mycobacterium tuberculosis*, the causative agent of TB [21]. Furthermore, their ability to induce apoptosis and inhibit cancer cell proliferation has sparked interest in their potential as anticancer agents [19].

The rationale for this study stems from the need to develop novel molecular hybrids that can overcome microbial resistance and improve anticancer efficacy. The emergence of multidrug-resistant bacterial and fungal strains has significantly reduced the effectiveness of conventional antibiotics, necessitating the search for new antimicrobial agents [22-24]. The design of isatin-chalcone derivatives as hybrid molecules offers a promising strategy for targeting key biomolecular pathways in microbial and cancer cells, potentially leading to enhanced therapeutic efficacy. The novelty of this research lies in the synthesis of structurally diverse isatin-chalcone derivatives aimed at improving their antimicrobial and anticancer potential. The synthesized compounds will be assessed against selected bacterial and fungal strains to determine their antimicrobial potential, while their anticancer efficacy will be investigated using *in vitro* cytotoxicity assays against cancer cell lines.

EXPERIMENTAL

All chemicals used in the synthesis were procured from the standard commercial sources. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel-G (Merck grade) with solvent systems specified where applicable. Column chromatography was performed using silica gel (100-200 mesh, Merck grade). Melting points were determined in open capillaries using a Digimelt melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a BRUKER AVANCE NMR spectrophotometer at 500 MHz using TMS as an internal standard, with chemical shifts expressed in δ ppm. Mass spectra were recorded on an Agilent 6320 Ion Trap spectrometer employing positive ionization mode.

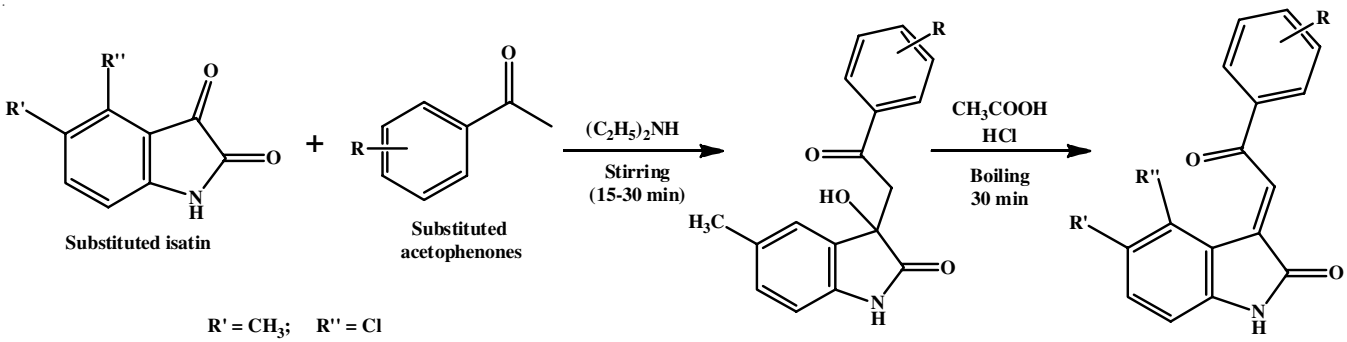
Synthesis of isatin-chalcone hybrids: A microwave assisted synthesis was employed to prepare the target isatin-chalcone hybrids. In a clean, dry beaker, a homogeneous mixture of 2 mmol of 5-methylisatin (or 4-chloroisatin) and 2 mmol of the appropriate acetophenone derivative was prepared. To this mixture, two drops of diethylamine were added as a catalyst. The reaction mixture was then subjected to microwave irradiation in a microwave oven (800 W) for 1-2 min. During this process, a colourless solid began to form, indicating the progress of the reaction. The reaction progress was monitored by TLC using a mobile phase of ethyl acetate and hexane (1:3 v/v). Upon completion of the initial reaction, 3 mL of glacial acetic acid and 3 drops of conc. HCl were added to the precipitate. The mixture was then returned to the microwave oven and irradiated for an additional 2-3 min to ensure complete cyclization and product formation. After the reaction was complete, the mixture was cooled to room temperature and ice-cold water (approximately 20 mL) was added to the reaction mixture to precipitate the product. The resulting solid was collected by vacuum filtration and washed thoroughly with distilled water (10 mL) to remove any residual acids and diethylammonium acetate byproducts (**Scheme-I**). The crude product was dried under reduced pressure and recrystallized with ethanol as solvent. For compounds that did not yield sufficiently pure products *via* recrystallization, column chromatography was employed using silica gel (60-120 mesh) and ethyl acetate/hexane gradient (1:4 to 1:2 v/v) as eluent [25].

(*E*)-5-Methyl-3-(2-oxo-2-phenylethylidene)indolin-2-one (Ia): Brick red solid, yield: 74.6%, m.p: 277-279 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.27 (N-H *str.*), 1779.08 (C=O, lactam), 1658.31 (C=O, conj. ketone), 1602.19 (C=C *str.*), 1322.26 (C-C *str.*), 1236.95 (C-N *str.*), 1014.78 (C-C vibr.), 810.73 (arom. C-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.71 (s, 1H), 8.09-8.07 (d, *J* = 8.0 Hz, 2H), 7.77-7.60 (m, 4H), 7.19-7.17 (d, *J* = 7.6 Hz, 1H), 6.80-6.78 (d, *J* = 8.0 Hz, 1H), 7.88 (s, 1H), 2.23 (s, 3H). ESI MS (*m/z*): 264.10 (M+H)$^+$.

(*E*)-5-Methyl-3-(2-oxo-2-(*p*-tolyl)ethylidene)indolin-2-one (Ib): Orange solid, yield: 71.9%, m.p: 301-303 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.30 (N-H *str.*), 1779.04 (C=O, lactam), 1659.03 (C=O, conj. ketone), 1607.92 (C=C *str.*), 1357.60 (C-N *str.*), 810.14 (arom. C-H bend.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.69 (s, 1H), 7.99-7.97 (d, *J* = 8.0 Hz, 2H), 7.68 (s, 1H), 7.53 (s, 1H), 7.43-7.41 (d, *J* = 7.6 Hz, 2H), 7.18-7.16 (d, *J* = 7.6 Hz, 1H), 6.80-6.78 (d, *J* = 7.6 Hz, 1H), 2.42 (s, 3H), 2.22 (s, 3H). ESI MS (*m/z*): 278.10 (M+H)$^+$.

(*E*)-3-(2-(4-Methoxyphenyl)-2-oxoethylidene)-5-methylindolin-2-one (Ic): Orange solid, yield: 72.2%, m.p: 323-325 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.11 (N-H *str.*), 1779.43 (C=O, lactam), 1654.20 (C=O, conj. ketone), 1605.64 (C=C *str.*), 1357.88 (C-N *str.*), 811.38 (arom. C-H bend.), 448.74 (C-C def.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.68 (s, 1H), 8.07-8.05 (d, *J* = 7.2 Hz, 2H), 7.82 (s, 1H), 7.68 (s, 1H), 7.17-7.14 (t, *J* = 9.2 Hz, 1H), 6.79-6.77 (d, *J* = 8.0 Hz, 1H), 3.88 (s, 3H), 2.22 (s, 3H); ESI MS (*m/z*): 294.20 (M+H)$^+$.

(*E*)-3-(2-(2-Fluorophenyl)-2-oxoethylidene)-5-methylindolin-2-one (Id): Red solid, yield: 75.6%, m.p: 290-292



R' = CH_3; R'' = Cl

R = -H (IA); -4-CH_3 (IB); -4-OCH_3 (IC); -2-F (ID); -3,4-OCH_3 (IE); -2-Cl (IF); -4-Cl (IG); -4-Br (IH); -4-F (II); -3-Br (IJ); -4-NH_2 (IK); -2-F (IL); -4-NO_2 (IM)

Scheme-I: Synthesis of new isatin linked chalcones from substituted isatins

ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.15 (N-H *str.*), 1779.18 (C=O, lactam), 1658.63 (C=O, conj. ketone), 1613.41 (C=C *str.*), 1358.63 (C-N *str.*), 1318.17 (C-F *str.*), 811.44 (arom. C-H bend.), 474.96 (C-C def.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.72 (s, 1H), 8.05 (s, 1H), 7.95-7.93 (t, *J* = 7.6 Hz, 1H), 7.75-7.74 (d, *J* = 6.0 Hz, 1H), 7.51 (s, 1H), 7.44-7.42 (d, *J* = 8.0 Hz, 1H), 7.21-7.19 (d, *J* = 7.6 Hz, 1H), 6.81-6.79 (dd, *J* = 7.6 Hz, 1H), 6.72-6.70 (dd, *J* = 8.0 Hz, 1H), 2.26 (s, 3H); ESI MS (*m/z*): 282.05 $(M+H)^+$.

(*E*)-3-(2-(3,4-Dimethoxyphenyl)-2-oxoethylidene)-5-methylindolin-2-one (Ie): Pale orange solid, yield: 70.8%, m.p: 369-371 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.13 (N-H *str.*), 1778.89 (C=O, lactam), 1656.38 (C=O, conj. ketone), 1602.76 (C=C *str.*), 1356.38 (C-N *str.*), 1269.79 (C-O *str.*), 810.48 (arom. C-H bend), 474.06 (C-C def.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.69 (s, 1H), 7.89 (s, 1H), 7.76-7.74 (t, *J* = 8.4 Hz, 2H), 7.58 (s, 1H), 7.17-7.15 (t, *J* = 8.0 Hz, 2H), 6.79-6.77 (d, *J* = 7.6 Hz, 1H), 3.88-3.87 (d, *J* = 4.4 Hz, 6H), 2.23 (s, 3H); ESI MS (*m/z*): 324.15 $(M+H)^+$.

(*E*)-4-Chloro-3-(2-(2-chlorophenyl)-2-oxoethylidene)-indolin-2-one (If): Yellow solid, yield: 79 %, m.p: 339-341 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.19 (N-H *str.*), 1779.39 (C=O, lactam), 1721.21 (C=O, keto), 1612.58 (C=C *str.*), 1358.35 (C-N *str.*), 808.81 (arom. C-H bend), 447.71 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H); ESI MS (*m/z*): 318.05 $(M+H)^+$.

(*E*)-4-Chloro-3-(2-(4-chlorophenyl)-2-oxoethylidene)-indolin-2-one (Ig): Pale yellow solid, yield: 74%, m.p: 339-341 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.10 (N-H *str.*), 1779.40 (C=O, lactam), 1721.21 (C=O, keto), 1643.08 (C=O, enol), 1615.64 (C=C *str.*), 1384.96 (C-N *str.*), 808.35 (arom. C-H bend.), 448.46 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H). ESI MS (*m/z*): 319.05 $(M+H)^+$.

(*E*)-3-(2-(4-Bromophenyl)-2-oxoethylidene)-4-chloro-indolin-2-one (Ih): Pale yellow solid, yield: 72.2%, m.p: 369-371 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.07 (N-H *str.*), 1779.07 (C=O, lactam), 1720.87 (C=O, keto), 1613.15 (C=C *str.*), 1324.84 (C-N *str.*), 807.67 (arom. C-H bend.), 473.81 (C-Br *str.*), 447.64 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H). ESI MS (*m/z*): 364.55 $(M+H)^+$.

(*E*)-4-Chloro-3-(2-(4-fluorophenyl)-2-oxoethylidene)-indolin-2-one (Ii): Pale yellow solid, yield: 74.6%, m.p: 309-311 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.50 (N-H *str.*), 1779.59 (C=O, lactam), 1720.62 (C=O, keto), 1616.40 (C=C *str.*), 1332.02 (C-N *str.*), 1150.20 (C-F *str.*), 808.52 (arom. C-H bend.), 427.21 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H). ESI MS (*m/z*): 303.05 $(M+H)^+$.

(*E*)-3-(2-(3-Bromophenyl)-2-oxoethylidene)-4-chloro-indolin-2-one (Ij): Pale yellow solid, yield: 70.8%, m.p: 369-371 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.49 (N-H *str.*), 1779.31 (C=O, lactam), 1724.87 (C=O, keto), 1612.80 (C=C *str.*), 1320.03 (C-N *str.*), 810.23 (arom. C-H bend.), 472.91 (C-Br *str.*), 448.20 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H); ESI MS (*m/z*): 364.55 $(M+H)^+$.

(*E*)-3-(2-(4-Aminophenyl)-2-oxoethylidene)-4-chloro-indolin-2-one (Ik): Dark yellow solid, yield: 68.4%, m.p: 392-394 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3350.14 (NH_2 *str.*), 1779.42 (C=O, lactam), 1707.47 (C=O, keto), 1642.49 (C=O, enol), 1332.13 (C-N *str.*), 1229.42 (C-N amine), 808.75 (arom. C-H bend.), 448.48 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H); ESI MS (*m/z*): 300.05 $(M+H)^+$.

(*E*)-4-Chloro-3-(2-(2-fluorophenyl)-2-oxoethylidene)-indolin-2-one (Il): Pale yellow solid, yield: 70.9, %, m.p: 309-311 ºC. FT-IR (KBr, ν_{max}, cm^{-1}): 3344.93 (N-H *str.*), 1779.01 (C=O, lactam), 1719.07 (C=O, keto), 1613.41 (C=C *str.*), 1333.24 (C-N *str.*), 834.73 (arom. C-H bend), 732.98 (C-F *str.*), 447.71 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H). ESI MS (*m/z*): 303.05 $(M+H)^+$.

(*E*)-4-Chloro-3-(2-(4-nitrophenyl)-2-oxoethylidene)-indolin-2-one (Im): Pale yellow solid, yield: 72.2%, m.p: 341-343 ºC, FT-IR (KBr, ν_{max}, cm^{-1}): 3345.00 (N-H *str.*), 1779.13 (C=O, lactam), 1718.29 (C=O, keto), 1608.47 (C=C *str.*), 1384.96 (CH def.), 1331.28 (N=O sym.), 1237.20 (N=O asym.), 797.68 (arom. C-H bend.), 448.48 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H), 8.01 (s, 1H), 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.55 (m, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.34-7.30 (t, *J* = 8.0 Hz, 1H), 7.10-7.08 (d, *J* = 8.0 Hz, 1H), 6.86-6.84 (d, *J* = 7.6 Hz, 1H). ESI MS (*m/z*): 330.05 $(M+H)^+$.

Biological evaluation

Antimicrobial activity: The synthesized isatin-linked chalcones were evaluated for antibacterial activity using the cup plate method. The activity was tested against Gram-positive (*Staphylococcus aureus* NCIM-2079) and Gram-negative (*Escherichia coli* NCIM-2068) bacteria, with amikacin as standard drug and DMSO as solvent and control. The micro-organisms were obtained from Biotechnology Lab, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam and maintained on nutrient agar medium.

The nutrient agar medium was prepared by dissolving 0.5% peptone, 0.3% beef extract, 1.5% agar and 0.5% sodium chloride in distilled water. The pH was adjusted to 6.8 and the medium was sterilized by autoclaving at 121 ºC (15 psi) for

20 min. Stock solutions (2 mg/mL) of the synthesized compounds and amikacin were prepared in DMSO. Serial dilutions were made to achieve concentrations of 20, 40, 60, 80, 100, 150 and 200 µg/mL for the test compounds. A bacterial suspension with an optical density (OD_{535} nm) of 0.6-0.8 was prepared and 100 µL of suspension was added to the sterilized nutrient agar medium. The inoculated medium was poured into sterilized petri plates and allowed to solidify for 15 min. Wells (10 mm diameter, 2 cm apart) were made in the agar using a sterile cork borer. Next, 100 µL of each concentration (20, 40, 60, 80, 100, 150 and 200 µg/mL) of the test compounds, amikacin (standard) and DMSO (control) were added to separate wells using a micropipette. The plates were incubated at 37 ºC for 24 h. After incubation, the antibacterial activity was assessed by measuring the zone of inhibition around each well [26].

Antifungal activity: Pure cultures of the experimental fungi, *Candida albicans* (NCIM 652) and *Aspergillus niger* (NCIM 3102), were obtained from Biotechnology Lab, Andhra University College of Pharmaceutical Sciences, Visakhapatnam. Stock solutions (2 mg/mL) of the synthesized compounds and fluconazole (standard drug) were prepared in DMSO. Serial dilutions were made to achieve concentrations of 50, 100 and 200 µg/mL for the test compounds. Sabouraud Dextrose Agar (SDA) medium was used for the isolation, cultivation and maintenance of fungal species. The medium was prepared by dissolving 10 g peptone, 40 g dextrose and 20 g agar in 1000 mL of distilled water. The mixture was sterilized by autoclaving at 121 ºC (15 psi) for 20 min.

The antifungal activity of the synthesized compounds was evaluated using the cup plate method. A 100 µL of fungal suspension (*C. albicans* and *A. niger*) was added to the sterilized Sabouraud's agar medium, which was then poured into sterilized petri plates. After solidification, wells (10 mm diameter, 2 cm apart) were prepared in the agar using a sterile cork borer. A 50 µL volume of each concentration (50, 100 and 200 µg/mL) of the test compounds, Fluconazole (standard) and DMSO (control) was added to separate wells. The plates were incubated at 26 ºC for 72 h. After incubation, the antifungal activity was assessed by measuring the zone of inhibition around each well [26].

Antitubercular activity: The antitubercular activity of the synthesized compounds (**IA-IM**) was evaluated against *Mycobacterium tuberculosis* H37Rv using the microplate Alamar blue assay (MABA). This method utilizes resazurin, a non-fluorescent blue dye that is reduced to pink, highly fluorescent resorufin in the presence of metabolically active cells. The reduction of resazurin serves as an indicator of cell viability and correlates well with the BACTEC radiometric method. Rifampicin, pyrazinamide, ciprofloxacin and streptomycin were used as standard drugs for comparison.

M. tuberculosis H37Rv strain was cultured on Lowenstein-Jensen (LJ) medium and the growth was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin-dextrose-catalase) enrichment. A 1:20 dilution of this suspension was used as inoculum for the MABA. All procedures were conducted in a biosafety hood to ensure sterility and safety. A 96-well microplate was prepared by adding 200 µL of sterile deionized water to the outer perimeter wells to minimize evaporation during incubation. In the test wells, 100 µL of Middlebrook 7H9 broth was added and serial dilutions of the test compounds were prepared directly on the plate to achieve final concentrations of 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg/mL. The plates were covered, sealed with parafilm and incubated at 37 ºC for 5 days. After incubation, 25 µL of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 was added to each well and the plates were incubated for an additional 24 h. A change in colour from blue to pink indicated bacterial growth, while wells retaining the blue colour were interpreted as having no growth. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented the colour change from blue to pink, indicating inhibition of *M. tuberculosis* growth [27].

Anticancer activity: The anticancer activity of synthesized compounds (**IA-IM**) was evaluated using the MTT assay on MCF-7 (breast cancer), HeLa (cervical cancer) and HEK-293 (embryonic kidney) cell lines obtained from ATCC. Frozen cell lines were revived by thawing in a 37 ºC water bath and transferring to pre-warmed DMEM with 10% FBS. After centrifugation at 1,500 rpm for 5 min, the cell pellet was resuspended in fresh DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were cultured in T-75 flasks at 37 ºC in a 5% CO_2 humidified incubator. Upon reaching 80-90% confluency, cells were washed with PBS, trypsinized using 0.25% trypsin-EDTA and resuspended in fresh medium. Cell viability was assessed using the trypan blue exclusion assay. Cells were counted using a hemocytometer or automated cell counter and seeded into 96-well plates at a density of 1×10^4 cells/well in 100 µL of DMEM. Plates were incubated at 37 ºC with 5% CO_2 for 24 h to allow cell attachment. Test compounds (**IA-IM**) were dissolved in DMSO (final concentration ≤ 0.1%) to prepare a 10 mM stock solution, followed by serial dilutions in DMEM to achieve working concentrations (1-100 µM). After 24 h, the medium was replaced with 100 µL of fresh medium containing the test compounds. After treatment, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well and plates were incubated for 4 h at 37 ºC in dark. The medium was then carefully removed and 100 µL of DMSO was added to dissolve the formazan crystals. Plates were gently shaken for 10 min and absorbance was measured at 570 nm (reference wavelength: 630 nm) using a microplate reader (BioTek, USA). Cell viability was calculated and IC_{50} values were determined using GraphPad Prism 9. All the experiments were performed in triplicate, with data expressed as mean ± SD. Safety protocols, including biosafety cabinet use and proper waste disposal, were followed [28].

RESULTS AND DISCUSSION

The isatin-chalcone hybrids were synthesized *via* microwave assisted reaction of 5-methylisatin (or 4-chloroisatin) and acetophenone derivatives using diethylamine as catalyst. The hybrids were obtained in good yield and high purity, confirmed by spectroscopic analysis.

Antibacterial activity: The antibacterial activity of the synthesized isatin-chalcone hybrids (**IA-IM**) against *S. aureus* and *E. coli* were evaluated using the cup plate method, with amikacin (4 µg) as standard.The zone of inhibition (ZOI) was measured at varying concentrations (20-200 µg) and the results are given in Tables 1 and 2.

All compounds exhibited a concentration-dependent increase in antibacterial activity. For example, compound **IK** (4-NH_2) showed a ZOI of 11 mm at 20 µg, which increased to 18 mm at 200 µg, surpassing the activity of amikacin (16 mm). Similarly, compounds **IF** (2-Cl) and **IL** (2-F) achieved ZOIs of 16 mm and 18 mm, respectively, at 200 µg, indicating potent antibacterial effects. Among the tested compounds, **IK** (4-NH_2), **IF** (2-Cl) and **IL** (2-F) demonstrated the highest activity, with ZOIs comparable to or exceeding that of Amikacin at higher concentrations. Compounds **IJ** (3-Br), **II** (4-F) and **IG** (4-Cl) showed moderate activity, with ZOIs ranging from 13-15 mm at 200 µg. In contrast, compounds **IA** (-H), **IB** (4-CH_3), **IC** (4-OCH_3), **ID** (2-F), **IE** (3,4-OCH_3) and **IM** (4-NO_2) exhibited lower activity, with ZOIs ranging from 8-12 mm at 200 µg.

The structure-activity relationship (SAR) analysis revealed that electron-donating groups, such as the amino group in **IK** (4-NH_2), significantly enhanced antibacterial potency. Halogen substitutions also influenced activity, with **IF** (2-Cl) and **IL** (2-F) showing superior activity compared to **IH** (4-Br) and **IJ** (3-Br). The position and type of halogen played a critical role in determining activity. Conversely, electron-withdrawing groups, such as the nitro group in **IM** (4-NO_2), reduced activity, suggesting that such groups may hinder antibacterial efficacy.

While amikacin (4 µg) consistently produced a ZOI of 16 mm, several test compounds (*e.g.* **IK**, **IF** and **IL**) achieved comparable or superior activity at higher concentrations (150-200 µg). This suggests that these hybrids have the potential to be further optimized for enhanced efficacy at lower concentrations. The results demonstrate that the isatin-chalcone hybrids exhibit significant antibacterial activity against *Staphylococcus aureus*, with compounds **IK** (4-NH_2), **IF** (2-Cl) and **IL** (2-F) showing particularly promising results. The concentration-dependent activity and structural insights provide a foundation for further optimization of these hybrids as potential antibacterial agents.

All compounds exhibited a concentration-dependent increase in antibacterial activity against *E. coli*. For example, compound **IM** (4-NO_2) showed a ZOI of 12 mm at 20 µg, which increased to 18 mm at 200 µg, surpassing the activity of amikacin (17 mm). Similarly, compound **IL** (2-F) achieved a ZOI of 17

TABLE-1
ANTIBACTERIAL ACTIVITY RESULTS OF ISATIN-CHALCONE HYBRIDS AGAINST *Staphylococcus aureus*

Compound	Zone of inhibition (mm)							
	20 µg	40 µg	60 µg	80 µg	100 µg	150 µg	200 µg	Amikacin (4 µg)
IA	4	5	6	7	10	11	12	16
IB	3	4	5	6	7	7	8	16
IC	4	5	6	7	8	9	10	16
ID	6	7	8	9	9	10	11	16
IE	4	5	6	7	8	9	10	16
IF	7	9	10	11	13	14	16	16
IG	6	7	8	9	11	12	13	16
IH	6	7	8	9	10	11	12	16
II	8	9	10	11	12	13	14	16
IJ	10	11	12	13	13	14	15	16
IK	11	12	13	14	15	16	18	16
IL	7	8	9	10	11	12	18	16
IM	4	5	7	8	9	10	11	16

TABLE-2
ANTIBACTERIAL ACTIVITY RESULTS OF ISATIN-CHALCONE HYBRIDS AGAINST *Escherichia coli*

Compound	Zone of inhibition (mm)							
	20 µg	40 µg	60 µg	80 µg	100 µg	150 µg	200 µg	Amikacin (4 µg)
IA	10	11	12	13	14	15	16	17
IB	8	9	10	11	12	14	15	17
IC	8	10	11	12	13	14	15	17
ID	9	10	11	12	13	15	16	17
IE	6	7	8	9	10	13	14	17
IF	9	11	12	13	14	15	16	17
IG	9	10	11	12	13	14	15	17
IH	8	9	10	11	12	13	14	17
II	9	11	12	13	14	15	16	17
IJ	8	9	10	11	12	13	14	17
IK	9	10	11	12	13	14	15	17
IL	11	12	13	14	15	16	17	17
IM	12	13	14	15	16	17	18	17

mm at 200 µg, matching the activity of amikacin. Among the tested compounds, **IM** (4-NO_2) and **IL** (2-F) demonstrated the highest activity, with ZOIs comparable to or exceeding that of Amikacin at higher concentrations. Compounds **IA** (H), **ID** (2-F), **IF** (2-Cl) and **II** (4-F) showed moderate activity, with ZOIs ranging from 16-17 mm at 200 µg. In contrast, compounds **IB** (4-CH_3), **IC** (4-OCH_3), **IE** (3,4-OCH_3), **IG** (4-Cl), **IH** (4-Br), **IJ** (3-Br) and **IK** (4-NH_2) exhibited lower activity, with ZOIs ranging from 14-15 mm at 200 µg.

The structure-activity relationship (SAR) analysis revealed that electron-withdrawing groups, such as the nitro group in **IM** (4-NO_2), significantly enhanced antibacterial potency. Halogen substitutions also influenced activity, with **IL** (2-F) and **IF** (2-Cl) showing superior activity compared to **IH** (4-Br) and **IJ** (3-Br). The position and type of substituent played a critical role in determining activity. Conversely, electron-donating groups, such as the methoxy group in **IC** (4-OCH_3) and **IE** (3,4-OCH_3), reduced activity, suggesting that such groups may hinder antibacterial efficacy against *E. coli*.

While amikacin (4 µg) consistently produced a ZOI of 17 mm, several test compounds (*e.g.* **IM**, **IL** and **IA**) achieved comparable or superior activity at higher concentrations (150-200 µg). This suggests that these hybrids have the potential to be further optimized for enhanced efficacy at lower concentrations. The results demonstrate that the isatin-chalcone hybrids exhibit significant antibacterial activity against *Escherichia coli*, with compounds **IM** (4-NO_2) and **IL** (2-F) showing particularly promising results.

Antifungal activity: The antifungal activity of the synthesized isatin-chalcone hybrids (**IA-IM**) was evaluated against *C. albicans* and *A. niger* using the cup-plate method, with fluconazole (4 µg) as the standard. The zone of inhibition was measured at concentrations of 50, 100 and 200 µg and the results are shown in Table-3.

Among the tested compounds, **IF** (2-Cl), **II** (4-F), **IK** (4-NH_2), **IL** (2-F) and **IM** (4-NO_2) exhibited significant antifungal activity, while the remaining compounds (**IA**-**IE**, **IG**-**IH**, **IJ**) showed no activity (NA) against both fungal strains. Compound **IM** (4-NO_2) demonstrated the highest activity against *A. niger*, with a ZOI of 17 mm at 200 µg, closely approaching the activity of fluconazole (18 mm). Similarly, **IK** (4-NH_2) and **IL** (2-F) showed promising activity against *C. albicans*, with ZOIs of 16 mm and 12 mm, respectively, at 200 µg. Compounds **IF** (2-Cl) and **II** (4-F) also displayed moderate activity, with ZOIs ranging from 13-14 mm at 200 µg against both fungal strains. In contrast, **IJ** (3-Br) exhibited lower activity, with ZOIs of 12 mm and 13 mm at 200 µg against *C. albicans* and *A. niger*, respectively.

The structure-activity relationship (SAR) analysis revealed that electron-withdrawing groups, such as the nitro group in compound **IM** (4-NO_2), significantly enhanced antifungal potency. Halogen substitutions also played a critical role, with compounds **IF** (2-Cl) and **II** (4-F) showing superior activity compared to compounds **IH** (4-Br) and **IJ** (-3-Br). The position and type of substituent influenced activity, with *o*-substituted compounds (*e.g.* **IF** (2-Cl) and **IL** (2-F)) generally exhibiting higher activity than *para*-substituted analogues. Conversely, the electron-donating groups, such as methoxy group in compounds **IC** (4-OCH_3) and **IE** (3,4-OCH_3), resulted in no activity, suggesting that such groups may hinder antifungal efficacy.

While fluconazole (4 µg) consistently produced a ZOI of 18 mm, several test compounds (*e.g.* **IM**, **IK** and **IL**) achieved comparable or near-comparable activity at higher concentrations (200 µg). This suggests that these hybrids have the potential to be further optimized for enhanced efficacy at lower concentrations. The results demonstrate that the isatin-chalcone hybrids exhibit significant antifungal activity against *C. albicans* and *A. niger*, with compounds **IM** (4-NO_2), **IK** (4-NH_2) and **IL** (2-F) showing particularly promising results. The concentration-dependent activity and structural insights provide a foundation for further optimization of these hybrids as potential antifungal agents.

Antitubercular activity: The antitubercular activity of the synthesized isatin-linked chalcones (**IA-IM**) was evaluated against *M. tuberculosis* H37Rv using the microplate Alamar blue assay (MABA). The activity was assessed at concentrations ranging from 0.78 to 25 µg/mL, with rifampicin used as standard drug. The results were enumerated in Table-4 categorized as

TABLE-3
ANTIFUNGAL ACTIVITY RESULTS OF ISATIN-CHALCONE HYBRIDS

Compound	Zone of inhibition (mm)						
	Candida albicans			*Aspergillus niger*			Flucanazole (4 µg)
	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg	
IA	NA	NA	NA	NA	NA	NA	18
IB	NA	NA	NA	NA	NA	NA	18
IC	NA	NA	NA	NA	NA	NA	18
ID	NA	NA	NA	NA	NA	NA	18
IE	NA	NA	NA	NA	NA	NA	18
IF	11	12	13	11	13	14	18
IG	NA	NA	NA	NA	NA	NA	18
IH	NA	NA	NA	NA	NA	NA	18
II	12	13	14	11	12	14	18
IJ	9	11	12	7	8	12	18
IK	11	12	16	7	12	13	18
IL	10	11	12	12	15	16	18
IM	10	11	13	15	16	17	18

TABLE-4
ANTITUBERCULAR ACTIVITY RESULTS OF ISATIN-CHALCONE HYBRIDS

Compound	Concentration (μg/mL)					
	25	12.5	6.25	3.12	1.56	0.78
IA	S	R	R	R	R	R
IB	R	R	R	R	R	R
IC	S	R	R	R	R	R
ID	S	S	R	R	R	R
IE	S	S	R	R	R	R
IF	S	S	R	R	R	R
IG	S	S	R	R	R	R
IH	S	R	R	R	R	R
II	S	R	R	R	R	R
IJ	S	R	R	R	R	R
IK	S	R	R	R	R	R
IL	S	R	R	R	R	R
IM	S	R	R	R	R	R
Rifampicin (standard)	S	S	S	S	R	R

TABLE-5
ANTICANCER ACTIVITY DATA (IC_{50}) OF ISATIN-CHALCONE HYBRIDS AGAINST DIFFERENT CELL LINES

Compound	Breast cancer (MCF-7)	Cervical cancer (HeLa)	Human embryonic kidney cell (HEK-293)
IA	21.37 ± 1.11	16.99 ± 1.12	30.81 ± 3.22
IB	17.51 ± 1.12	17.89 ± 1.95	38.85 ± 1.07
IC	17.83 ± 2.58	15.02 ± 1.68	35.94 ± 1.62
ID	11.58 ± 0.91	12.69 ± 1.22	34.12 ± 4.52
IE	11.94 ± 1.05	9.47 ± 1.12	27.47 ± 3.05
IF	13.86 ± 2.10	14.94 ± 1.21	32.45 ± 2.01
Ig	16.61 ± 1.45	15.16 ± 1.41	28.03 ± 4.52
IH	6.68 ± 1.06	10.15 ± 1.99	33.07 ± 2.44
II	9.38 ± 1.11	13.33 ± 1.12	32.82 ± 1.25
Ij	15.31 ± 1.12	14.75 ± 1.95	38.85 ± 0.91
Ik	6.53 ± 1.12	10.91 ± 3.02	34.52 ± 1.57
IL	18.93 ± 3.37	14.94 ± 1.21	35.19 ± 1.13
IM	21.12 ± 2.27	14.19 ± 3.84	38.56 ± 1.57
Doxorubicin	2.76 ± 0.87	3.89 ± 0.62	4.03 ± 1.12

sensitive (S) or resistant (R) based on the inhibition of bacterial growth.

All the compounds (**IA-IM**) exhibited sensitivity at the highest concentration tested (25 μg/mL), indicating significant antitubercular activity at this dose. Among these, compounds **ID**, **IE**, **IF** and **IG** showed enhanced activity, demonstrating sensitivity at both 25 μg/mL and 12.5 μg/mL. This suggests that these compounds possess structural features that improve their efficacy against *M. tuberculosis*. For instance, compounds **ID** (2-F), **IF** (2-Cl) and **IG** (4-Cl) contain halogen substitutions, which may enhance interactions with bacterial targets. Similarly, compound **IE** (-3,4-OCH_3) includes electron-donating methoxy groups, which could also contribute to its activity. In contrast, the remaining compounds (**IA**, **IB**, **IC**, **IH**, **II**, **IJ**, **IK**, **IL**, **IM**) were sensitive only at 25 μg/mL, indicating moderate activity. Rifampicin, the standard drug, showed sensitivity at concentrations up to 3.12 μg/mL, highlighting its superior potency compared to the synthesized compounds.

The structure-activity relationship (SAR) analysis revealed that halogen substitutions and electron-donating groups play a significant role in enhancing antitubercular activity. However, none of the compounds demonstrated activity at concentrations below 6.25 μg/mL, indicating a threshold for effective inhibition. This suggests that further structural optimization is needed to improve potency and reduce the minimum effective concentration. The results demonstrate that the isatin-linked chalcones, particularly compounds **ID**, **IE**, **IF** and **IG**, exhibit promising antitubercular activity and provide a foundation for future modifications to develop more effective analogues.

Anticancer activity: The cytotoxic potential of the tested compounds (**IA-IM**) was evaluated against breast cancer (MCF-7), cervical cancer (HeLa) and human embryonic kidney (HEK-293) cell lines. The IC_{50} values (Table-5) were used to assess the potency and selectivity of the compounds compared to the standard chemotherapeutic agent doxorubicin.

Breast cancer (MCF-7) cell line: Compounds **IH** (4-Br) and **IK** (4-NH_2) exhibited the highest potency, with IC_{50} values of 6.68 ± 1.06 μM and 6.53 ± 1.12 μM, respectively. These values are significantly lower than those of other compounds, indicating strong cytotoxicity against breast cancer cells. Compounds **ID** (2-F), **IE** (-3,4-OCH_3) and **II** (4-F) also showed notable activity, with IC_{50} values ranging from 9.38 ± 1.11 μM to 11.94 ± 1.05 μM. In contrast, compounds **IA** (H), **IB** (4-CH_3) and **IM** (4-NO_2) were the least potent, with IC_{50} values exceeding 17 μM, suggesting reduced efficacy. The structure-activity relationship (SAR) analysis revealed that electron-donating groups (*e.g.*, -NH_2 in **IK**) and halogen substitutions (*e.g.*, -Br in **IH**) enhance activity, while electron-withdrawing groups (*e.g.*, -NO_2 in **IM**) reduce potency.

Cervical cancer (HeLa) cell line: Against the HeLa cervical cancer cell line, compounds **IE** (-3,4-OCH_3) and **IH** (4-Br) demonstrated the highest cytotoxicity, with IC_{50} values of 9.47 ± 1.12 μM and 10.15 ± 1.99 μM, respectively. Compounds **ID** (2-F), **IF** (2-Cl) and **IK** (4-NH_2) also showed strong activity, with IC_{50} values ranging from 10.91 ± 3.02 μM to 14.94 ± 1.21 μM. On the other hand, compounds **IA** (-H), **IB** (4-CH_3) and **IM** (4-NO_2) exhibited lower potency, with IC_{50} values above 14 μM. The SAR analysis indicated that electron-donating groups (*e.g.*, -OCH_3 in **IE**) and halogen substitutions (*e.g.*, -Br in **IH**) play a critical role in enhancing activity against cervical cancer cells. The high selectivity of **IE** (-3,4-OCH_3), with an IC_{50} of 9.47 ± 1.12 μM for HeLa cells and 27.47 ± 3.05 μM for HEK-293 cells, highlights its potential as a selective anticancer agent.

Human embryonic kidney (HEK-293) cell line: The cytotoxicity of the synthesized compounds against the HEK-293 normal cell line was evaluated to assess selectivity. Most compounds exhibited IC_{50} values significantly higher than those for cancer cell lines, indicating lower toxicity toward normal cells. For example, **IE** (3,4-OCH_3) showed an IC_{50} of 27.47 ± 3.05 μM, while **IH** (4-Br) and **IK** (4-NH_2) had IC_{50} values of 33.07 ± 2.44 μM and 34.52 ± 1.57 μM, respectively. In contrast, doxorubicin, the standard drug, exhibited low selectivity, with an IC_{50} of 4.03 ± 1.12 μM for HEK-293 cells, which is close to its IC_{50} values for cancer cells. This highlights the superior selectivity of the synthesized compounds, particularly compounds

IE, **IH** and **IK**, which preferentially target cancer cells while sparing normal cells. The results demonstrate that the isatin-chalcone hybrids exhibit significant anticancer activity, with compounds **IH** (4-Br), **IK** (4-NH_2) and **IE** (3,4-OCH_3) showing particularly promising potency and selectivity across breast and cervical cancer cell lines. These compounds provide a foundation for further optimization to develop more effective and selective anticancer agents.

The selectivity index analysis revealed important insights into the anticancer potential of the synthesized compounds. Highly potent compounds such as compounds **IE**, **IH** and **IK**, exhibited low IC_{50} values against cancer cell lines, particularly HeLa, indicating strong cytotoxic effects. In contrast, compounds **IB**, **IM** and **IA** were the least potent across all cell lines, showing higher IC_{50} values and reduced efficacy. Among the tested compounds, **IE** demonstrated the highest selectivity, with a low IC_{50} of 9.47 µM for HeLa cells and a significantly higher IC_{50} of 27.47 µM for HEK-293 cells, suggesting preferential toxicity toward cancer cells while sparing normal cells. In comparison, doxorubicin, although the most potent, displayed lower selectivity, as its IC_{50} values for both normal and cancer cells were in a similar range. These findings highlight the potential of compound **IE** as a promising anticancer agent with improved selectivity over conventional chemotherapeutic drugs.

Overall, compounds **IE**, **IH** and **IK** exhibited the most promising anticancer activity, particularly against breast and cervical cancer cell lines, with moderate selectivity over normal cells. Compound **IE** demonstrated the best balance between potency and selectivity, making it a potential lead compound for further investigations. The findings indicate that certain tested compounds could serve as effective alternatives to doxorubicin with potentially reduced toxicity to normal cells, warranting further indepth mechanistic studies.

Conclusion

A series of novel isatin-chalcone hybrids were synthesized *via* microwave-assisted methods and evaluated for antimicrobial, antifungal, antitubercular and anticancer activities. Compounds **IK** (4-NH_2), **IF** (2-Cl) and **IL** (2-F) exhibited potent antibacterial activity against *S. aureus* and *E. coli*, with zones of inhibition (ZOI) comparable to or exceeding amikacin at higher concentrations. Against *C. albicans* and *A. niger*, compounds **IM** (4-NO_2), **IK** (4-NH_2) and **IL** (2-F) showed significant antifungal activity, approaching fluconazole's efficacy. In antitubercular assays, compounds **ID** (2-F), **IE** (-3,4-OCH_3), **IF** (2-Cl) and **IG** (4-Cl) demonstrated sensitivity at 12.5–25 µg/mL against *M. tuberculosis*. Anticancer evaluations revealed that compounds **IH** (4-Br), **IK** (4-NH_2) and **IE** (-3,4-OCH_3) were highly potent against breast (MCF-7) and cervical (HeLa) cancer cells, with IC_{50} values as low as 6.53 ± 1.12 µM, while sparing normal HEK-293 cells. Structure-activity relationship (SAR) studies highlighted the importance of electron-donating groups (*e.g.* -NH_2, -OCH_3) and halogen substitutions (*e.g.* -Cl, -Br) in enhancing activity. These findings underscore the potential of isatin-chalcone hybrids as multifunctional therapeutic agents, warranting further optimization and development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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