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Microwave Assisted Synthesis of Some New 2-(4-Substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles of Biological Interest

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A novel series of imidazo-indole hybrid compounds, focusing on their antibacterial, anticancer and anthelmintic efficacy was designed and synthesized. All the compounds, 2-(4-substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles (**1b-10b**) were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. Conventional heating and microwave irradiation were both used in the synthesis process, although the microwave irradiation provided better efficiency, faster response times and safer environmental conditions. The synthesized compounds were screened for their antibacterial activity against Gram-positive and Gram-negative pathogens, revealing promising efficacy in several compounds, particularly **2b**, **5b** and **6b**. Furthermore, their anticancer potential was evaluated using the EAC cell line approach, with compounds **1b**, **2b**, **4b** and **5b** exhibiting excellent activity, with CTC_{50} values of 31.25 µg/mL, 51.61 µg/mL, 44.21 µg/mL and 31.25 µg/mL, respectively. Additionally, most compounds also displayed moderate to good anthelmintic activity, highlighting their potential as therapeutic agents.

Keywords: Imidazo-indole hybrid compounds, Microwave synthesis, Antibacterial activity, Anticancer activity, Anthelmintic activty.

INTRODUCTION

Imidazole is a 5-membered aromatic ring with two nitrogen atoms positioned at the non-adjacent locations. This structure imparts significant electronic versatility, allowing imidazole containing compounds to engage in various chemical interactions [1-4]. Its properties are harnessed in the design of pharmaceuticals, including antifungal agents and enzyme inhibitors. Similarly, indole, consisting of a fused benzene and pyrrole ring, is also renowned for its biological significance. The indole moiety is a core structure in many natural products and pharmaceuticals, such as serotonin and various alkaloids [5-9]. Its electron rich nature and capacity to participate in π-π stacking interactions make it valuable in drug design and materials science.

The hybridization of imidazole and indole moieties into a single heterocyclic framework represents an advanced approach in the design of novel compounds with potentially enhanced biological and chemical properties [10-14]. These hybrid derivatives combine the structural and electronic characteristics of both imidazole and indole, offering a unique platform for the development of multifunctional molecules.

The multifactorial diseases like malaria, cancer, diabetic complications, Alzheimer's disease, inflammatory conditions, cardiovascular diseases, tuberculosis, *etc*. arises from an interplay of genetic and environmental factors, lacking a singular, definitive mechanism. Moreover, these diseases necessitate multi-target pharmacological treatments because single-target medications are ineffective. Therefore, the creation of hybrid molecules represents a robust approach that integrates two or more heterocyclic compounds with potent biological activity, facilitating their concurrent delivery to targeted organs. The advancements in long-term outcomes and reduced toxicity can be linked to this hybrid approach, which significantly differs from the conventional practice of co-administering separate target molecules [15-17].

In this work, we have synthesized imidazo-indole hybrid derivatives, *viz*. 2-(4-substituted phenyl)-3-(1*H*-indol-4-yl)-imidazo[4,5-*b*]indoles (**1b-10b**) by both conventional as well

as microwave assisted method. The synthesized compounds were further characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass, elemental analysis as well as evaluation of antibacterial, anti-cancer and anthelmintic efficacies.

EXPERIMENTAL

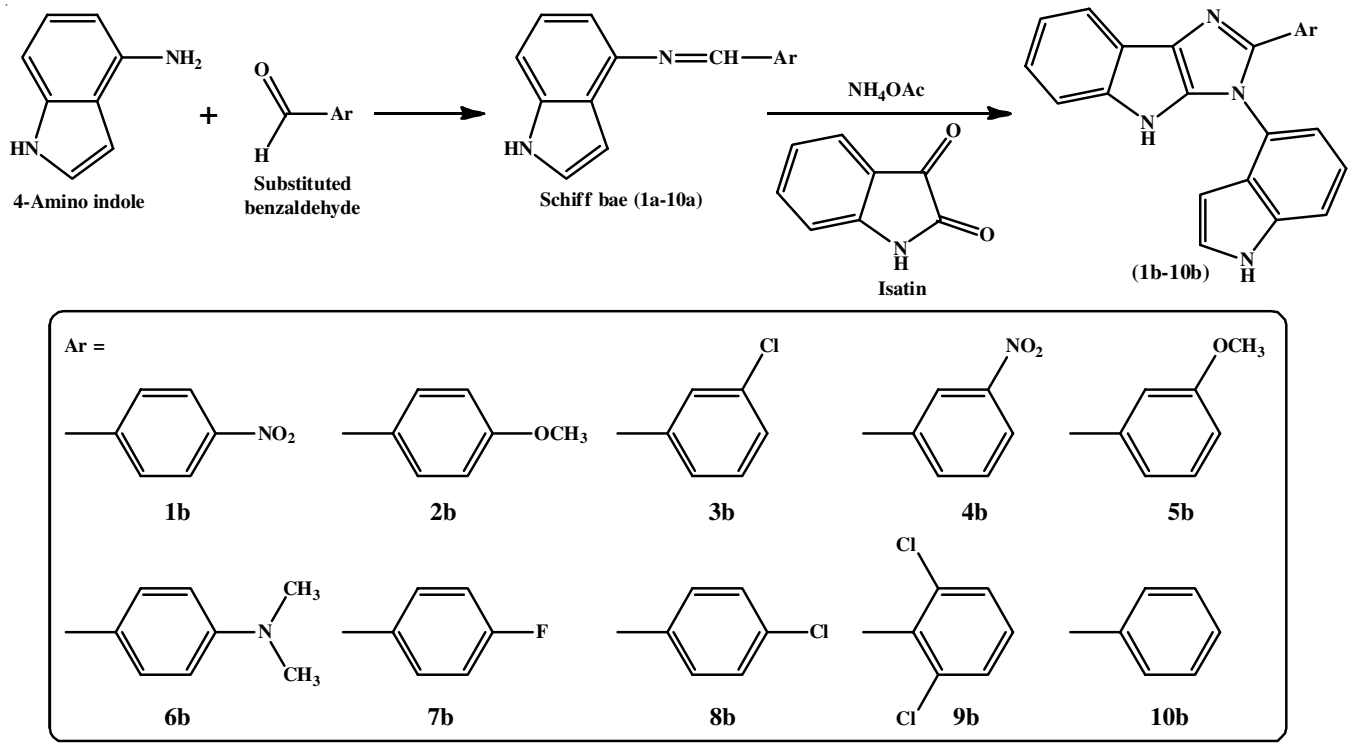
All the analytical grade chemicals used in this study were procured from commercial sources and employed without further purification. The melting points were measured in open capillary tubes and are corrected. FT-IR spectra were obtained using a Perkin-Elmer Infrared-283 spectrophotometer utilizing KBr pellets for sample preparation. Thin-layer chromatography (TLC) analysis was performed on aluminum plates coated with 0.25 mm thick silica gel (60 F_{254}). The NMR spectra for proton (^{1}H NMR) and carbon (^{13}C NMR) were recorded on a Bruker DRX-400 spectrometer operating at 400 MHz, utilizing FT NMR techniques. Tetramethylsilane (TMS) was used as an internal standard and the solvents for NMR analysis were chloroform-*d* ($CDCl_3$) and DMSO-d_6. The elemental analysis was performed using a Vario EL III instrument from Elementar and mass spectra were acquired with a Shimadzu LC-MS 2010AT spectrometer.

Conventional method for synthesis of Schiff bases (1a-10a): In a 500 mL flat-bottom flask, a reaction mixture having 4-aminoindole (0.01 M) and a substituted aromatic aldehyde (0.01 M) was prepared in 25 mL glacial acetic acid. The mixture was heated and refluxed for 6 h. Reaction progress was tracked using TLC analysis. After completion, the mixture was cooled to ambient temperature, yielding the crude Schiff base product from the condensation of 4-aminoindole and aromatic aldehyde. This intermediate was subsequently utilized in the synthesis of imidazo-indole hybrids without additional purification steps.

Conventional method for synthesis of 2-(4-substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles (1b-10b): The reaction mixture consisting of isatin (0.01 M), ammonium acetate (0.1 M) and Schiff base (0.01 M) was mixed thoroughly and then refluxed for 9-10 h. The reaction progress was monitored using TLC. After the reaction completion, the mixture was poured into 150 mL of ice-cold water, the solid product was separated by filtration and then washed thoroughly with benzene (35 mL). The product was then recrystallized from chloroform to obtain the substituted analogues of imidazo-indoles (**1b-10b**) (**Scheme-I**).

Microwave method for synthesis of Schiff bases (1a-10a): A homogeneous mixture was prepared by triturating 4-amino-indole (0.01 M) and substituted aromatic aldehyde (0.01 M) in a dry and clean mortar. This mixture was then divided among four separate 100 mL beakers, each containing 5 g of activated silica gel. The beakers were subjected to microwave irradiation for approximately 7 min at a power setting of 1000 W. Throughout the irradiation process, the samples underwent periodic cooling intervals every 60 s to prevent overheating. To ensure homogeneous heating, the reaction mixtures were vigorously stirred and periodically cooled. Following the TLC verification of reaction completion, the resultant products were employed directly in the subsequent synthesis of imidazo-indole derivatives without additional purification.

Microwave method for synthesis of 2-(4-substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles (1b-10b): In a dry and clean mortar, a mixture of isatin (0.01 M), ammonium acetate (0.1 M) and Schiff base (0.01 M) was triturated to develop



Scheme-I: 2-(4-Substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles (**1b-10b**)

a uniform slurry and then transferred into a 100 mL beaker. Similarly, other reaction mixtures were prepared and placed in separate beakers. The reaction mixtures were exposed to microwave energy at 1000 W for 11-15 min, with intermittent cooling pulses every 60 s to control the thermal accumulation. Simultaneously, vigorous stirring and cooling steps maintained uniform temperature conditions.

Once the reaction was completed, as confirmed by TLC, the mixtures were transferred into separate 250 mL beakers containing ice-cold water. This step was performed to remove excess acetic acid and ammonium acetate byproducts. The precipitated solids were then isolated *via* filtration and then washed thoroughly with 25 mL of benzene to eliminate the residual isatin, followed by recrystallization from chloroform to yield the desired substituted imidazo-indole analogues (**1b-10b**) (**Scheme-I**).

3-(1*H*-Indol-4-yl)-2-(4-nitrophenyl)imidazo[4,5-*b*]-indole (1b): Caramel-brown; m.p.: 192 ºC; IR Elemental analysis of calcd. (found) % of $C_{23}H_{15}N_5O_2$: C, 70.22 (70.28); H, 3.84 (3.78); N, 17.80 (17.72); O, 8.13 (8.29). (KBr, ν_{max}, cm^{-1}): 3345 (Ar N-H), 3049 (Ar C-H), 1666 (C=C), 1570 (C=N), 1450 (N=O), 1264 (C-N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.38 (1H, d, *J* = 2.4, CH), 7.01-7.79 (10H, m, Ar-H), 8.28 (2H, dd, *J* = 2.8, CH), 10.08 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 101.6, 110.9, 110.6, 112.9, 114.4, 119.3, 120.5 (2C), 120.9, 121.2 (2C), 121.9, 123.9, 124.6, 128.8 (2C), 129.4, 135.1, 136.2 (2C), 137.3, 145.0, 148.6; EIMS (*m/z*): $[M]^+$ 393.87; 354.71, 347.88, 278.92, 272.04, 123.36, 116.8, 67.65.

3-(1*H*-Indol-4-yl)-2-(4-methoxyphenyl)imidazo[4,5-*b*]-indole (2b): Off-white; m.p.: 176 ºC; Elemental analysis of calcd. (found) % of $C_{24}H_{18}N_4O$: C, 76.17 (76.15); H, 4.79 (4.75); N, 14.81 (14.93); O, 4.23 (4.16). IR (KBr, ν_{max}, cm^{-1}): 3357 (Ar N-H), 3066 (Ar C-H), 1654 (C=C), 1552 (C=N), 1220 (C-O-C), 1255 (C-N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 3.82 (3H, s, OCH_3), 6.47 (1H, d, *J* = 2.4, CH), 6.91 (2H, dd, *J* = 3.2, CH), 7.09-7.63 (10H, m, Ar-H), 10.18 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 56.0, 102.6, 110.9, 111.3, 112.1, 113.6, 114.3 (2C), 118.2, 120.6 (2C), 120.9, 121.7, 122.6, 123.7, 124.1, 128.2 (2C), 128.9, 134.7, 136.5 (2C), 145.3, 161.2; EIMS (*m/z*): $[M]^+$ 378.97; 348.83, 339.14, 272.86, 263.04, 117.60, 108.48, 67.44.

2-(3-Chlorophenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]-indole (3b): Saffron; m.p.: 220 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{15}N_4Cl$: C, 72.16 (72.27); H, 3.95 (3.91); Cl, 9.26 (9.32); N, 14.63 (14.59). IR (KBr, ν_{max}, cm^{-1}): 3329 (Ar N-H), 3092 (Ar C-H), 1650 (C=C), 1555 (C=N), 1260 (C-N), 746 (C-Cl); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.48 (1H, d, *J* = 2.4, CH), 7.01-7.70 (12H, m, Ar-H), 10.10 (2H, s, NH); ^{13}C NMR(DMSO-d_6, 400 MHz) δ ppm: 101.8, 111.1, 111.6, 112.1, 114.5, 118.7, 120.2 (2C), 120.8, 122.7, 124.5, 124.9, 125.4, 127.8, 129.1, 129.9, 130.6, 131.9, 134.6, 135.7, 136.1 (2C), 144.3; EIMS (*m/z*): $[M]^+$ 382.95, $[M+2]^+$ 384.8, 348.15, 344.27, 272.93, 267.29, 116.81, 112.91, 67.60.

3-(1*H*-Indol-4-yl)-2-(3-nitrophenyl)imidazo[4,5-*b*]-indole (4b): Pale yellow; m.p.: 192 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{15}N_5O_2$: C, 70.22 (70.38); H, 3.84 (3.78); N, 17.80 (17.93); O, 8.13 (8.17). IR (KBr, ν_{max}, cm^{-1}): 3355 (Ar N-H), 3055 (Ar C-H), 1656 (C=C), 1550 (C=N), 1447 (N=O), 1256 (C-N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.44 (1H, d, *J* = 2.4, CH), 6.99-7.75 (10H, m, Ar-H), 8.22 (1H, d, *J* = 2.0, CH), 8.40 (1H, s, CH), 10.11 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 101.7, 109.8, 110.7, 112.8, 114.2, 118.8, 120.4 (2C), 120.9, 121.6, 122.4, 122.6, 124.5, 124.9, 128.1, 130.4, 131.9, 133.1, 135.1, 136.3 (2C), 145.1, 148.2; EIMS (*m/z*): $[M]^+$ 393.78, 354.52, 347.76, 278.92, 272.04, 123.42, 116.8, 67.48.

3-(1*H*-Indol-4-yl)-2-(3-methoxyphenyl)imidazo[4,5-*b*]-indole (5b): Ivory crystals; m.p.: 176 ºC; Elemental analysis of calcd. (found) % of $C_{24}H_{18}N_4O$: C, 76.17 (76.04); H, 4.79 (4.82); N, 14.81 (14.77); O, 4.23 (4.32). IR (KBr, ν_{max}, cm^{-1}): 3354 (Ar N-H), 3056 (Ar C-H), 1655 (C=C), 1565 (C=N), 1254 (C-N), 1217 (C-O-C); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 3.78 (3H, s, OCH_3), 6.41 (1H, d, *J* = 3.2, CH), 6.76 (1H, d, *J* = 2.8, CH), 6.88 (1H, s, CH), 6.99-7.65 (10H, m, Ar-H), 10.09 (2H, s, CH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 55.6, 102.6, 110.1, 110.8, 111.7, 112.2, 113.8 (2C), 118.8, 119.4, 120.4 (2C), 121.6, 122.2, 124.7, 124.9, 128.4, 130.1, 132.0, 135.6, 136.1 (2C), 145.2, 161.4; EIMS (*m/z*): $[M]^+$ 378.84, 348.71, 339.34, 272.86, 263.26, 117.45, 108.62, 67.32.

4-(3-(1*H*-Indol-4-yl)imidazo[4,5-*b*]indol-2-yl)-*N*,*N*-dimethylbenzenamine (6b): Golden yellow; m.p.: 205 ºC; Elemental analysis of calcd. (found) % of $C_{25}H_{21}N_5$: C, 76.70 (76.75); H, 5.41 (5.39); N, 17.89 (17.90). IR (KBr, ν_{max}, cm^{-1}): 3356 (Ar N-H), 3052 (Ar C-H), 1628 (C=C), 1546 (C=N), 1274 (C-N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 2.92 (6H, s, $N(CH_3)_2$], 6.46 (1H, d, *J* = 2.4, CH), 6.68 (2H, dd, *J* = 2.8, CH), 7.11-7.63 (10H, m, Ar-H), 10.19 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 40.8 (2C), 102.6, 110.8, 111.4, 112.1, 114.1, 114.9 (2C), 118.6, 120.4 (2C), 120.4, 121.3, 122.6, 124.6, 124.9, 128.1 (2C), 128.6, 135.1, 136.6 (2C), 145.1, 149.8; EIMS (*m/z*): $[M]^+$ 392.03, 352.78, 348.82, 276.6, 272.04, 121.53, 116.73, 67.45.

2-(4-Fluorophenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]-indole (7b): Wine red; m.p.: 195 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{15}N_4F$: C, 75.40 (75.44); H, 4.13 (4.06); F, 5.19 (5.12); N, 15.29 (15.25). IR (KBr, ν_{max}, cm^{-1}): 3332 (Ar N-H), 3075 (Ar C-H), 1653 (C=C), 1560 (C=N), 1235 (C-N), 792 (C-F); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.39 (1H, d, *J* = 2.4, CH), 6.99-7.65 (12H, m, Ar-H), 10.07 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm:102.7, 110.9, 111.4, 112.4, 114.1, 116.5 (2C), 119.4, 120.4 (2C), 121.7, 122.1, 124.2, 124.6, 126.1, 128.1, 129.5 (2C), 135.1, 136.1 (2C), 144.3, 162.5; EIMS (*m/z*): $[M]^+$ 366.89, 348.72, 327.07, 272.78, 250.98, 117.51, 96.48, 66.81.

2-(4-Chlorophenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]-indole (8b): Dull yellow; m.p.: 192 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{15}N_4Cl$: C, 72.16 (72.23); H, 3.95 (3.93); Cl, 9.26 (9.29); N, 14.63 (14.60). IR (KBr, ν_{max}, cm^{-1}): 3323 (Ar N-H), 3090 (Ar C-H), 1625 (C=C), 1552 (C=N), 1268 (C-N), 755 (C-Cl); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.38 (1H, d, *J* = 2.8, CH), 7.03-7.60 (12H, m, Ar-H), 10.09 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 102.9, 110.7, 111.7, 112.3, 113.2, 118.5, 120.3 (2C), 120.8, 121.6, 124.5, 124.9, 128.4, 128.9, 129.3 (2C), 129.9 (2C), 133.9, 134.8, 136.1 (2C),

145.1; EIMS (*m/z*): $[M]^+$ 382.88, $[M+2]^+$ 384.53, 348.38, 344.46, 272.93, 267.18, 116.81, 112.68, 67.52.

2-(2,6-Dichlorophenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]-indole (9b): light yellow; m.p.: 203 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{14}Cl_2N_4$: C, 66.20 (66.32); H, 3.38 (3.33); Cl, 16.99 (17.02); N, 13.43 (13.39). IR (KBr, ν_{max}, cm^{-1}): 3342 (Ar N-H), 3089 (Ar C-H), 1625 (C=C), 1540 (C=N), 1265 (C-N), 775 (C-Cl); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.43 (1H, d, *J* = 2.8, CH), 7.02-7.60 (11H, m, Ar-H), 10.13 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 102.9, 110.8, 110.7, 111.8, 113.6, 118.7, 129.9 (2C), 121.2, 122.1, 124.6, 125.3, 127.1 (2C), 128.4, 130.8, 133.6 (2C), 135.1, 136.8 (2C), 137.6, 145.6; EIMS (*m/z*): $[M]^+$ 416.82, $[M+2]^+$ 418.6, 348.05, 309.65, 301.74, 272.64, 147.42, 117.51, 67.55.

3-(1*H*-Indol-4-yl)-2-phenylimidazo[4,5-*b*]indole (10b): Off-white; m.p.: 145 ºC; Elemental analysis of calcd. (found) % of $C_{23}H_{16}N_4$: C, 79.29 (79.36); H, 4.63 (4.61); N, 16.08 (16.03). IR (KBr, ν_{max}, cm^{-1}): 3354 (Ar N-H), 3032 (Ar C-H), 1652 (C=C), 1558 (C=N), 1248 (C-N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ ppm: 6.39 (1H, d, *J* = 2.4, CH), 7.01-7.68 (13H, m, Ar-H), 10.14 (2H, s, NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 101.9, 109.8, 111.4, 112.7, 114.1, 120.2 (2C), 121.5, 122.1, 124.6, 124.9, 127.2 (2C), 128.1, 128.7, 129.1 (2C), 130.2, 135.4, 136.1 (2C), 145.1 ; EIMS (*m/z*): $[M]^+$ 348.86, 309.82, 271.87, 233.14, 117.47, 78.57, 67.52.

Biological studies

Antibacterial activity: In this study, the agar disk diffusion method was performed applying a microbial inoculum of approximately 1-2 × 10^8 CFU/mL to a 150 mm solid agar plate. Filter paper discs (12 mm in diameter) were placed on the agar surface and antimicrobial agents were added in various concentrations. The selected microorganisms was then inoculated onto the agar plates, followed by the placement of 6 mm filter paper discs. The inoculated petri dishes containing test compound and the microorganisms were incubated at 27 ºC. The zone of inhibition, representing the area where microbial growth was inhibited, was carefully measured to the nearest millimeter.

Anticancer activity: The synthesized compounds were screened for anticancer activity using the Trypan blue dye exclusion test [18-20], following established protocols. The compounds were tested at five dose levels: 500, 250, 125, 62.5 and 31.25 µg/mL and their ability to inhibit EAC cell line growth was calculated. 6-Mercaptopurine was used as the standard drug, with a CTC_{50} value of 31.25 µg/mL. The growth inhibition percentage was determined using eqn. 1:

$$\text{Growth inhibition } (\%) = \frac{\text{Total cell} - \text{Live cell}}{\text{Total cell}} \times 100$$

To calculate the CTC_{50}, a graphical representation of concentration *versus* percentage growth inhibition was constructed and the concentration yielding 50% inhibition was extrapolated.

Anthelmintic studies: The anthelmintic properties of the compounds were assessed against two earthworm species, *Pontoscolex corethrurus* (ICARBC 408) and *Megascoplex konkanensis* (ICARBC 211) by following reported method [21,22] at the concentration of 2 mg/mL. Sample suspensions were prepared by grinding 100 mg of synthesized compounds with 0.5% Tween 80 and distilled water followed by mechanical stirring for 30 min. The suspensions were then diluted to achieve 0.2% w/v concentration of the test samples. A standard anthelmintic drug, albendazole was used as reference. Three replicates of five earthworms (~5 cm in length) were exposed to either test samples or reference drug suspensions (50 mL) in 10 cm Petri dishes at ambient temperature. A control group consist of 50 mL distilled water with 0.5% Tween 80. Paralysis and mortality times were documented and mean values were calculated from the triplicate experiments. To ascertain mortality, earthworms were immersed in warm water (50 ºC), eliciting the movement in living individuals.

RESULTS AND DISCUSSION

The synthesis of 2-(4-substituted phenyl)-3-(1*H*-indol-4-yl)imidazo[4,5-*b*]indoles (**1b-10b**) containing imidazo-indole scaffold was successfully accomplished. The FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrum and elemental analysis were used to confirm the structures of all the newly synthesized compounds. The IR spectroscopic data of compounds **1b-10b** showed the specific absorption signatures, providing structural insights into their molecular framework. The spectra displayed aromatic C-H stretching vibrations at 3092-3032 cm^{-1}, C-O-C stretching vibrations at 1220-1217 cm^{-1} and C-Cl stretching vibrations at 775-746 cm^{-1}. Moreover, the presence of C-F bonds was confirmed by absorption at 792 cm^{-1}. The IR spectra also revealed characteristic absorption bands for C=C stretching (1666-1625 cm^{-1}), aromatic C=N stretching (1570-1540 cm^{-1}) and C-N stretching (1274-1248 cm^{-1}), further elucidating the molecular structure of the synthesized compounds.

The ^{1}H NMR spectra of the synthesized compounds (**1b-10b**) revealed well-defined proton signals. Aromatic ring protons resonated in the range of 6.99-7.79 ppm (multiplet) and 6.38-6.48 ppm (doublet) for the indole ring. The substituted aryl group at the *para* position exhibited double doublets at 8.28, 6.91 and 6.68 ppm characteristic of nitro, methoxy and dimethylamino groups, respectively. Moreover, the methoxy group C-H protons appeared as singlets at 3.78 ppm (*meta*) and 3.82 ppm (*para*), while the C-H proton of dimethylamino group resonated as a singlet at 2.92 ppm, providing clear evidence of the structural features of the synthesized compounds. The ^{13}C NMR spectra of the synthesized compounds also displayed a range of signals corresponding to the aromatic ring carbons (101.6-161.4 ppm). Furthermore, specific signals were detected for the CH_3-O carbon (55.6 & 56.0 ppm), C-F carbon (162.5 ppm), CH_3-N carbon (40.8 ppm) and C-NO_2 carbon (148.2 & 148.6 ppm), allowing for the unambiguous assignment of functional groups and structural features in the compounds.

The mass spectral data and elemental analysis results were also in accordance with the proposed structures of all the synthesized compounds. The compounds bearing the chlorine (Cl) group exhibited [M+2] peaks in their mass spectra, providing further confirmation of their structural assignments.

Antibacterial activity: The *in vitro* antimicrobial activity of the synthesized compounds (**1b-10b**) was investigated against the Gram-positive *Streptococcus aureus* and *Bacillus subtilis*

as well as the Gram-negative *Escherichia coli* and *Klebsiella pneumoniae*. Tetracycline was employed as the reference compound, exhibiting a zone of inhibition diameter. The majority of the tested compounds exhibited moderate antibacterial activity against all the studied bacterial strains. However, compounds **2b, 5b** and **6b** demonstrated superior activity, resembling the inhibition efficacy of the standard bactericide against most of the Gram-positive and Gram-negative bacteria tested (Table-1). It is suggested that the presence of electron-donating groups, such as dimethylamino group at position 4 (**6b**) and methoxy group in the substituted phenyl group at positions 3 and 4 (**5b** and **2b**) is crucial for the antibacterial potency.

Anticancer activity: The anticancer potential of the synthesized compounds was evaluated *in vitro* against the EAC cell line. Table-1 displays the CTC_{50} values, representing the concentration at which 50% cell growth inhibition was achieved. Compounds **1b**, **2b**, **4b** and **5b** showed moderate anticancer activity at CTC_{50} values 31.25, 31.25, 44.41 and 50.61 μg/mL, respectively under *in vitro* anticancer screening using EAC cell line in comparison to standard 6-mercaptopurine showing CTC_{50} value at 31.25 μg/mL. The nitro group, with its electronegative properties, plays a key role in increasing the potency of compounds **1b** and **4b**. Similarly, the methoxy group in compounds **2b** and **5b** also has a positive impact on their potential.

Anthelmintic activity: The newly synthesized imidazole-indole hybrid derivatives exhibited a promising anthelmintic efficacy, demonstrating moderate to good activity at a concentration of 2 mg/mL in Tween 80 (0.5%)-distilled water solution. Remarkably, the synthesized compounds displayed superior potency against *P. corethruses* and *M. konkanensis* compared to the reference standard, albendazole (Table-2). The combination of imidazole and indole moieties may result in a synergistic effect, leading to increased potency or a broader spectrum of activity against diverse helminth species.

Conclusion

In summary, a series of imidazo-indole hybrid compounds is synthesized both conventional as well as microwave assisted methods. All characterized compounds have shown significant promising antimicrobial, anticancer and anthelmintic agents.

TABLE-1
DATA OF SYNTHESIZED IMIDAZO-INDOLE HYBRID DERIVATIVES WERE SUBJECTED TO BIOLOGICAL SCREENING AGAINST EAC (EHRLICH ASCITES CARCINOMA) CELL LINES AND VARIOUS ANTIBACTERIAL STRAINS

Compd.	Reaction time		Yield (%)		CTC_{50}[c] (μg/mL)	Diameter of zone of inhibition (mm) Antimicrobial strains			
	MW[a] (min)	Conven.[b] (h)	MW (min)	Conven. (h)		Gram-positive		Gram-negative	
						B. subtilis	*S. aureus*	*K. pneumoniae*	*E. coli*
1b	13	05	83	54	31.25	9.3 (50.0)	10.6 (25.0)	11.9 (50.0)	12.7 (25.0)
2b	11	06	87	52	31.25	7.9 (6.25)	8.3 (12.5)	9.6 (12.5)	7.8 (6.25)
3b	12	08	90	48	100.21	9.4 (25.0)	7.9 (50)	9.6 (50.0)	9.8 (25.0)
4b	13	05	85	49	44.41	7.6 (50.0)	8.2 (100.0)	9.6 (100.0)	12.5 (50.0)
5b	12	06	84	58	50.61	10.8 (6.25)	11.6 (6.25)	9.4 (12.5)	12.1 (6.25)
6b	15	07	82	50	100.61	11.2 (6.25)	10.9 (12.5)	8.9 (12.5)	11.1 (12.5)
7b	11	09	88	54	100.21	10.1 (25.0)	9.2 (50.0)	8.2 (100)	8.2 (50.0)
8b	12	08	85	53	205.50	8.5 (50.0)	10.2 (100)	10.6 (50.0)	10.6 (100)
9b	10	10	86	51	91.61	9.8 (50.0)	9.6 (25.0)	8.2 (25.0)	9.5 (100)
10 b	12	09	87	53	101.25	9.6 (50.0)	9.1 (100)	8.2 (25.0)	9.6 (50.0)
Std. Drug	Anticancer (6-mercaptopurine) Antibacterial (tetracycline)				31.25	12 (6.25)	15 (12.5)	16 (12.5)	12 (6.25)

[a]Microwave irradiation, [b]Conventional, [c]Cytotoxic concentration (which inhibited 50% of total cells)

TABLE-2
DATA OF ANTHELMINTIC ACTIVITY ALL SYNTHESIZED IMIDAZO-INDOLE HYBRID DERIVATIVES SCREENED AGAINST VARIOUS EARTH WORM SPECIES

Compound	*P. corethruses*		*M. konkanensis*	
	Mean paralyzing time (min)*	Mean death time (min)*	Mean paralyzing time (min)*	Mean death time (min)*
1b	12.14 ± 0.85	19.18 ± 1.18	13.47 ± 1.45	22.02 ± 1.08
2b	11.22 ± 0.36	18.56 ± 0.71	14.65 ± 1.22	21.22 ± 1.21
3b	11.66 ± 1.46	20.22 ± 0.94	13.98 ± 1.16	23.76 ± 0.42
4b	11.28 ± 0.61	20.03 ± 1.30	13.76 ± 0.51	21.72 ± 0.45
5b	12.50 ± 0.34	19.12 ± 0.87	14.83 ± 1.41	21.53 ± 1.61
6b	11.95 ± 0.45	20.23 ± 1.61	13.92 ± 0.65	21.31 ± 0.67
7b	11.96 ± 0.12	19.34 ± 0.75	13.67 ± 0.57	19.34 ± 0.46
8b	10.91 ± 0.26	16.12 ± 0.78	16.91 ± 0.45	21.50 ± 0.56
9b	12.56 ± 0.45	20.16 ± 0.46	14.65 ± 1.23	22.19 ± 0.35
10b	12.31 ± 1.16	20.36 ± 1.15	15.12 ± 0.23	23.19 ± 0.56
Control	–	–	–	–
Albendazole	12.32 ± 0.67	20.16 ± 0.75	14.23 ± 1.19	22.54 ± 1.23

*Data are given as mean ± S.D (n = 3)

The presence of dimethylamino and methoxy groups enhanced antibacterial activity, while nitro and methoxy substitutions improved the anticancer efficacy. Additionally, most of the compounds exhibited moderate to good anthelmintic activity, highlighting their therapeutic potential. These results warrant further investigation to fully explore the possibilities of these compounds as novel therapeutic agents.

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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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