

Research Article



Microwave-Assisted and Parallel Synthesis of Some Novel Imidazoles as Anticancer and Anthelmintics

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ABSTRACT

In the present study we have made an attempt to synthesize novel imidazoles and evaluate them as potential therapeutic agents for anticancer and Anthelmintic activity. First, sulfanilamide was condensed with heteryl aldehydes to afford corresponding Schiff's base. The Schiff's base further on treatment with NH_4OAC and acetylated / benzoylated isatin using silica gel as solid support yielded corresponding imidazoles. In this paper a comparative study between the developed microwave method and conventional method is described. The synthesized compounds were analyzed by physical and analytical data. The synthesized compounds were evaluated for their Anthelmintic and short-term anticancer activity. All the synthesized substituted imidazoles have shown moderate to good Anthelmintic activity. The synthesized imidazoles possessed significant cytotoxic activity against HEP2 cell line by SRB assay.

Keywords: Anthelmintic activity, Anticancer activity, Imidazole, Microwave-assisted synthesis.

INTRODUCTION

The discovery of new compounds with anticancer activity has become one of the most important goals in medicinal chemistry. One of the most often used classes of chemotherapeutic agents in cancer therapy comprises molecules that interact with DNA, such as groove binders, DNA alkylating substances and intercalators. Moreover, the study of the exact mechanism of action of these agents, as well as, DNA damage of the cancer cells are of high interest for medicinal chemists and molecular biologists. For example, the intercalation of planar aromatic molecules into the DNA double helix and poisoning of DNA topoisomerases I and/or II are considered to be important in the therapeutic action of many anticancer agents.^{1,2}

Syntheses of heterocyclic compounds from readily available reagents by simple and efficient methods are the major requirements of heterocyclic chemistry. A survey of the pertinent literature reveals that, imidazole derivatives possess diverse biological activities apart from their synthetic interests. They are reported to exhibit pharmacological activities such as cognitive enhancers^{3,4}, anticancer⁵⁻⁸, antimicrobial^{9,10}, anthelmintic^{11,12} and anti-inflammatory activities.¹³ Some of the best selling therapies today contain this versatile heterocycle in their core structures. Therefore, it would be difficult to underestimate the importance of imidazole in the pharmaceutical industry.

In 1858, Debus reported the reaction between glyoxal and ammonia, ever since this reaction became a novel route to the syntheses of imidazoles.¹⁴ Later, a number of articles have described the syntheses of various imidazoles.¹⁵⁻²⁴

MATERIALS AND METHODS

Microwave irradiation was done using microwave oven supplied by Catalyst microwave synthesis system, Model: CATA-2R. Melting points were recorded on capillary melting point apparatus and are uncorrected. ^1H spectra were recorded on Bruker DRX-400 (400 MHz, FT NMR) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on LC-MS (Schimadzu-2010AT) under Electro Spray Ionization (ESI) technique. IR spectra (λ_{max} in cm^{-1}) were recorded on Perkin-Elmer infrared-283 FTIR spectrometer. Elemental analyses were recorded on Elementar Vario EL spectrometer. All chromatographic purification was performed with silica gel 60 (230–400 mesh), whereas all TLC (silica gel) development was performed on silica gel coated (Merck Kiesel 60 F254, 0.2 mm thickness) sheets. All chemicals were purchased from Aldrich Chemical Ltd (Milwaukee, WI, USA). Solvents used for the chemical synthesis acquired from commercial sources were of analytical grade, and were used without further purification unless otherwise stated.

In view of these observations and in continuation of our endeavor to develop better and potent anticancer and anthelmintic agents new series of novel imidazoles were synthesized as follows:

General Procedure for the Preparation of Schiff's Bases (1A- 8A)

Conventional Method

Equimolar amounts (0.01 M) of sulfanilamide and substituted aromatic aldehydes were transferred to a 250 ml round bottom flask containing 15 ml glacial acetic acid and refluxed for 6 h. The reaction mixture was allowed to cool to give product. The reactions were monitored



through TLC. The completed reactions were taken directly for the preparation of imidazoles.

Microwave Method

Equimolar amounts (0.01 M) of sulfanilamide and substituted aromatic aldehydes were transferred to a clean and dry mortar, triturated to form uniform mixture. The reaction mixture was then transferred to a 100 ml beaker containing 2 g of activated silica gel. All the beakers containing different reaction mixtures were also kept inside the microwave oven and then microwave irradiation was carried out at 1000 W for about 8 min. Intermittent cooling was done after every 60 sec of microwave irradiation. During intermittent cooling, the reaction mixtures were thoroughly mixed. The reactions were monitored through TLC. The products so obtained were taken directly for the preparation of novel imidazoles.

Procedure for Preparation of *N*-Acetylated Isatin (Scheme-I)

Isatin (0.01 mol), Acetyl chloride (0.01 mol) and pyridine (0.01 mol) were transferred into a 100 ml beaker. The reaction mixture was thoroughly mixed and kept in a microwave oven.

Microwave irradiation was done at 350 W for 3-4 minutes with intermittent cooling and mixing after every 1 minute. The completion of reaction was monitored through TLC using 10% methanol in chloroform and 1 to 2 drops of petroleum ether. (%Yield: 84.12, R_f : 0.889, Mp 193-194°C).

Procedure for Preparation of *N*-Benzoylated Isatin (Scheme-II)

Isatin (0.01 mol), Benzoyl chloride (0.01 mol) and Sodium hydroxide (0.01 mol) were transferred into a 100 ml beaker. The reaction mixture was thoroughly mixed and kept in a microwave oven.

Microwave irradiation was done at 350 W for 3-4 minutes with intermittent cooling and mixing after every 1 minute. The completion of reaction was monitored through TLC using 10% methanol in chloroform and 1 to 2 drops of petroleum ether. (%Yield: 83.94, R_f : 0.854, Mp - 184-185 °C).

General Procedure for the Preparation of Imidazoles By Solid Phase, Solvent Free Reaction (1B-16B) (Scheme I & II)

Conventional Method

N-Acetylated isatin / *N*-Benzoylated isatin (0.01M) was transferred along with excess of ammonium acetate (0.1 M) into a flask containing the Schiff's base (~0.01M) obtained by conventional procedure. The reaction mixture was stirred and refluxed on heating plate with magnetic stirrer for about 14-17 h. The reaction was monitored through TLC.

The reaction mixture was poured into 250 ml of water to remove ammonium acetate and acetic acid then it was filtered and dried in hot air oven. The crude product was washed with 2 x 20 ml of benzene to remove traces of any unreacted *N*-acetylated isatin / *N*-benzoylated isatin and products were recrystallized by ethyl acetate.

Microwave Method

This reaction was carried out in a parallel synthetic way as shown in Scheme 1. *N*-Acetylated isatin / *N*-Benzoylated isatin (0.01M) was transferred along with excess of ammonium acetate (0.1 M) in to a dry mortar containing the Schiff's base (~0.01M) obtained from the earlier microwave procedure. It was triturated to form a uniform mixture. The reaction mixture was then transferred to 100 ml beaker. Like this all other beakers containing different reaction mixtures were kept inside the microwave oven in a circle and then microwave irradiation was carried out at 1000 W for about 22-25 min. Intermittent cooling was done after every 60 sec of microwave irradiation. During intermittent cooling, the reaction mixtures were thoroughly mixed. The reactions were monitored through TLC. The reaction mixtures were withdrawn from microwave oven soon after the reaction is completed based on TLC data at regular intervals.

The completed and cooled reaction mixture was poured in to 250 ml of water to remove ammonium acetate and acetic acid, filtered and dried in hot air oven. The crude product was washed with 2 x 20 ml of benzene to remove traces of any un-reacted *N*-acetylated isatin / *N*-benzoylated isatin further extracted with ethyl acetate. The ethyl acetate was heated, filtered in hot condition and allowed to cool. The solid crystals formed were collected by filtration and dried under vacuum.

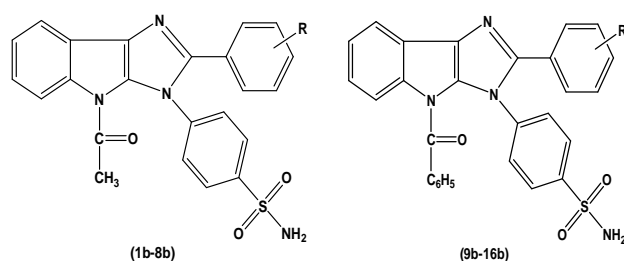
Anticancer Studies by SRB Assay

The anticancer activity was carried out in HEP₂ cell line using SRB assay method. SRB is a bright pink aminoxanthine dye with two sulphonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed color can be measured over a broad range of visible wavelength in 96 well plate readers. When TCA-fixed, SRB stained samples are air dried. They can be stored indefinitely without deterioration. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitreplate, 0.1 ml of the diluted suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and μ L of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37° for 3 days in 5% CO₂ atmosphere, and microscopic



examination was carried out and observations recorded every 24 hours. After 72 hours, 25 μL of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form a over all concentration of 10%. The plates were incubated at 4 $^{\circ}\text{C}$ for one h. The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum and were then air dried. The air dried plates were stained with Sulforhodamine B, a protein binding dye for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 μL of 10 Mm tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate readed at a wavelength at 540 nm. The percentage growth inhibition was calculated.^{25,26} The results are given in table 1.

Table 1: Data of All Synthesized Novel Imidazoles (1b-16b)



Compound No.	Reaction time		Yield (%) ^a		CTC ₅₀ ^c value ($\mu\text{g/ml}$)
	MW ^b (min)	Conven (h)	MW ^b (min)	Conven (h)	
1b	23.5	14.5	65.61	27.64	46.25
2b	25.0	15.5	77.64	31.82	32.41
3b	25.0	15.5	83.61	33.24	86.32
4b	24.0	15.0	74.67	29.42	93.45
5b	23.5	14.5	61.58	24.20	>150
6b	23.0	14.0	61.37	23.97	115.23
7b	23.5	15.0	63.94	26.21	38.41
8b	24.5	16.0	69.13	27.12	82.33
9b	23.5	15.0	83.91	35.60	97.41
10b	23.0	14.5	71.42	31.39	43.25
11b	24.5	15.0	82.51	36.99	74.22
12b	25.0	16.0	75.27	38.21	>150
13b	24.0	14.5	68.48	28.32	104.07
14b	24.5	15.5	62.98	25.20	69.13
15b	23.5	14.5	70.02	31.93	102.32
16b	24.5	16.5	74.01	32.89	34.46
5-Fluorouracil	-	-	-	-	31.76

a: Isolated yield; b: Microwave irradiation; c: The cytotoxic concentration (which inhibited 50 % of total cells).

Anthelmintic Studies

Anthelmintic activity studies were carried out against three different species of earthworms *M. konkanensis*, *P. corethruses* and *Eudrilus* at 2 mg ml⁻¹ concentration using Garg and Atal method.²⁷ Suspensions of samples were prepared by triturating synthesized compounds (100 mg) with Tween 80 (0.5%) and distilled water and the resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of the test samples. Suspension of reference drug, mebendazole, was prepared with the same concentration in a similar way. Three sets of five earthworms of almost similar sizes (2 inch in length) were placed in Petri plates of 4 inch diameter containing 50 ml of suspension of test sample and reference drug at RT. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and tween 80 (0.5%). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 $^{\circ}\text{C}$) which stimulated the movement, if the worm was alive. The results are given in table 2.

4-[2-(3'-Nitrophenyl)imidazo[4,5-b]N-acetylindole-3(4H)-yl]benzenesulfonamide (1b, C₂₃H₁₇N₅O₅S)

Mp ($^{\circ}\text{C}$): 70; Rf value: 0.77; IR (KBr): 3322.12 (N-H), 3082.04 (Ar C-H), 2921.96 (Ali C-H), 1665.02 (C=O), 1595.09 (C=N), 1546.22 (C=C), 1402.15 (NO₂), 1329.68 (S-N), 1151.42 (C-N), 1073.56 (S=O), 858.77 (C-N stretching for NO₂), 682.75 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 2.85 (s, 3H, CH₃), 6.51 (s, 2H, NH₂, D₂O exchangeable), 6.62-8.88 (m, 12H, Ar-H) ppm; ¹³C NMR: δ = 22.5, 110.1, 117.2, 119.0 (2), 120.1, 120.6 (2), 121.2 (2), 122.0, 126.1 (2), 127.1, 129.1, 129.9, 131.5, 134.2 (2), 142.4, 142.6, 146.7, 165.4 ppm; MS (ESI) m/z: M+1 peak found, 475.96 (M+1 peak calculated, 476.1); Anal. Calcd. for: C, 58.10; H, 3.60; N, 14.73; S, 6.74. Found: C, 58.15; H, 3.57; N, 14.70; S, 6.69.

4-[2-(2'-Hydroxyphenyl)imidazo[4,5-b]N-acetylindole-3(4H)-yl]benzenesulfonamide (2b, C₂₃H₁₈N₄O₄S)

Mp ($^{\circ}\text{C}$): 79; Rf value: 0.71; IR (KBr): 3570.42 (O-H), 3330.71 (N-H), 3080.17 (Ar C-H), 2889.17 (Ali C-H), 1654.24 (C=O), 1560.10 (C=N), 1546.22 (C=C), 1324.68 (S-N), 1195.78 (C-N), 1093.56 (S=O), 685.25 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 2.82 (s, 3H, CH₃), 6.90 (s, 2H, NH₂, D₂O exchangeable), 6.96-7.98 (m, 12H, Ar-H), 8.97 (s, 1H, OH, D₂O exchangeable) ppm; ¹³C NMR: δ = 22.6, 110.2, 115.2, 117.1 (2), 119.1 (2), 120.1, 121.4 (2), 121.9, 122.0, 126.2 (2), 127.1, 129.2, 133.7, 134.2, 137.2, 138.4, 142.4, 153.1, 166.1 ppm; MS (ESI) m/z: M+1 peak found, 447.01 (M+1 peak calculated, 447.10); Anal. Calcd. for: C, 61.87; H, 4.06; N, 12.55; S, 7.18. Found: C, 61.80; H, 4.12; N, 12.42; S, 7.12.

4-[2-(3'-Hydroxyphenyl)imidazo[4,5-b]N-acetylindole-3(4H)-yl]benzenesulfonamide (3b, C₂₃H₁₈N₄O₄S)

Mp (°C): 47; Rf value: 0.64; FTIR (KBr): 3559.77 (O-H), 3317.35 (N-H), 3091.68 (Ar C-H), 2956.67 (Ali C-H), 1658.42 (C=O), 1561.94 (C=N), 1556.34 (C=C), 1319.68 (S-N), 1149.50 (C-N), 1041.49 (S=O), 688.54 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 3.31 (s, 3H, CH_3), 6.35 (s, 2H, NH_2 , D_2O exchangeable), 6.71-8.43 (m, 12H, Ar-H), 8.84 (s, 1H, OH, D_2O exchangeable) ppm; ^{13}C NMR: δ = 22.5, 110.2, 111.2, 113.2, 117.1, 118.2 (3), 120.2, 120.4 (2), 122.0, 126.2 (2), 128.7, 130.1, 133.7, 134.4, 137.1, 138.3, 142.7, 157.0, 165.7 ppm; Anal. Calcd. for: C, 61.87; H, 4.06; N, 12.55; S, 7.18. Found: C, 61.77; H, 4.02; N, 12.43; S, 7.21.

4-[2-(2'-Chlorophenyl)imidazo[4,5-*b*]N-acetylindole-3(4*H*)-yl]benzenesulfonamide (4b, $\text{C}_{23}\text{H}_{17}\text{ClN}_4\text{O}_3\text{S}$)

Mp (°C): 62; Rf value: 0.73; FTIR (KBr): 3328.83 (N-H), 3063.62 (Ar C-H), 2923.88 (Ali C-H), 1675.24 (C=O), 1569.66 (C=N), 1543.76 (C=C), 1327.68 (S-N), 1151.42 (C-N), 1041.56 (S=O), 756.04 (C-Cl), 682.75 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 2.65 (s, 3H, CH_3), 6.72 (s, 2H, NH_2 , D_2O exchangeable), 6.90-8.87 (m, 12H, Ar-H) ppm; ^{13}C NMR: δ = 22.6, 110.1, 117.0, 118.2 (2), 120.6, 121.3 (2), 122.0, 125.3, 126.2 (3), 127.4, 128.2, 130.2, 133.7, 134.2, 136.5, 137.2, 138.6, 142.9, 165.5 ppm; MS (ESI) m/z: M+1 peak found, 465.02 (M+1 peak calculated, 465.07); Anal. Calcd. for: C, 59.42; H, 3.69; N, 12.05; S, 6.90. Found: C, 59.48; H, 3.71; N, 11.99; S, 6.83.

4-[2-(4'-Chlorophenyl)imidazo[4,5-*b*]N-acetylindole-3(4*H*)-yl]benzenesulfonamide (5b, $\text{C}_{23}\text{H}_{17}\text{ClN}_4\text{O}_3\text{S}$)

Mp (°C): 60; Rf value: 0.22; FTIR (KBr): 3332.82 (N-H), 3080.11 (Ar C-H), 2923.88 (Ali C-H), 1672.16 (C=O), 1594.73 (C=N), 1547.54 (C=C), 1328.68 (S-N), 1151.42 (C-N), 1008.70 (S=O), 752.19 (C-Cl), 676.97 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 3.36 (s, 3H, CH_3), 6.51 (s, 2H, NH_2 , D_2O exchangeable), 6.62-8.88 (m, 12H, Ar-H) ppm; ^{13}C NMR: δ = 22.5, 110.1, 117.0, 118.1 (2), 120.2, 120.7 (2), 122.0, 126.2 (2), 126.8 (3), 127.4 (2), 132.2, 133.5, 134.4, 137.2, 138.6, 142.8, 165.4 ppm; Anal. Calcd. for: C, 59.42; H, 3.69; N, 12.05; S, 6.90. Found: C, 59.40; H, 3.75; N, 11.99; S, 6.93.

4-[2-(3'-Chlorophenyl)imidazo[4,5-*b*]N-acetylindole-3(4*H*)-yl]benzenesulfonamide (6b, $\text{C}_{23}\text{H}_{17}\text{ClN}_4\text{O}_3\text{S}$)

Mp (°C): 67; Rf value: 0.79; FTIR (KBr): 3310.75 (N-H), 3095.18 (Ar C-H), 2925.81 (Ali C-H), 1668.24 (C=O), 1545.22 (C=C), 1521.73 (C=N), 1325.68 (S-N), 1151.42 (C-N), 1041.49 (S=O), 756.04 (C-Cl), 626.82 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 2.25 (s, 3H, CH_3), 6.51 (s, 2H, NH_2 , D_2O exchangeable), 6.62-8.88 (m, 12H, Ar-H) ppm; ^{13}C NMR: δ = 22.5, 110.1, 117.0, 118.1 (2), 120.2, 120.7 (2), 122.0, 123.6, 125.4, 126.2 (2), 126.8, 128.7, 130.1, 132.6 (2), 133.5, 137.2, 138.6, 142.7, 165.5 ppm; Anal. Calcd. for: C, 59.42; H, 3.69; N, 12.05; S, 6.90. Found: C, 59.38; H, 3.56; N, 11.99; S, 6.96.

4-[2-(4'-Nitrophenyl)imidazo[4,5-*b*]N-acetylindole-3(4*H*)-yl]benzenesulfonamide (7b, $\text{C}_{23}\text{H}_{17}\text{N}_5\text{O}_5\text{S}$)

Mp (°C): 55; Rf value: 0.72; FTIR (KBr): 3341.52 (N-H), 3074.32 (Ar C-H), 2925.81 (Ali C-H), 1664.24 (C=O),

1591.16 (C=N), 1546.22 (C=C), 1398.30 (NO_2), 1329.68 (S-N), 1151.42 (C-N), 1039.56 (S=O), 837.05 (C-N stretching for NO_2), 684.68 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 3.11 (s, 3H, CH_3), 6.41 (s, 2H, NH_2 , D_2O exchangeable), 6.62-8.81 (m, 12H, Ar-H) ppm; ^{13}C NMR: δ = 22.5, 110.1, 117.0, 118.1, 119.6 (2), 120.2, 120.7 (2), 122.0, 126.2 (2), 126.4 (3), 133.5, 134.4 (2), 137.2, 138.6, 142.2, 146.8, 165.5 ppm; Anal. Calcd. for: C, 58.10; H, 3.60; N, 14.73; S, 6.74. Found: C, 58.14; H, 3.62; N, 14.70; S, 6.78.

4-[2-(3'-Methoxyphenyl)imidazo[4,5-*b*]N-acetylindole-3(4*H*)-yl]benzenesulfonamide (8b, $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$)

Mp (°C): 51; Rf value: 0.50; FTIR (KBr): 3350.76 (N-H), 3072.39 (Ar C-H), 2925.81 (Ali C-H), 1658.12 (C=O), 1593.09 (C=N), 1547.22 (C=C), 1328.93 (S-N), 1236.29 (C-N), 1149.50 (C-O-C), 1039.56 (S=O), 690.47 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 3.31 (s, 3H, CH_3), 3.50 (s, 3H, OCH_3), 6.33 (s, 2H, NH_2 , D_2O exchangeable), 6.61-8.43 (m, 12H, Ar-H) ppm; ^{13}C NMR: δ = 22.6, 54.5, 109.1 (2), 112.3, 117.0, 117.8, 118.1, 120.2, 120.7 (2), 122.0, 126.2 (3), 128.3, 129.1, 133.5, 134.4, 137.2, 138.6, 142.8, 159.0, 165.6 ppm; MS (ESI) m/z: M+1 peak found, 460.98 (M+1 peak calculated, 461.12); Anal. Calcd. for: C, 62.60; H, 4.38; N, 12.17; S, 6.96. Found: C, 62.53; H, 4.39; N, 12.05; S, 6.93.

4-[2-(3'-Nitrophenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzenesulfonamide (9b, $\text{C}_{28}\text{H}_{19}\text{N}_5\text{O}_5\text{S}$)

Mp (°C): 62; Rf value: 0.59; FTIR (KBr): 3339.56 (N-H), 3096.12 (Ar C-H), 1653.02 (C=O), 1598.12 (C=N), 1546.22 (C=C), 1399.98 (NO_2), 1321.68 (S-N), 1094.49 (C-N), 1046.16 (S=O), 853.12 (C-N stretching for NO_2), 688.44 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 6.51 (s, 2H, NH_2 , D_2O exchangeable), 6.62-8.88 (m, 17H, Ar-H) ppm; ^{13}C NMR: δ = 110.1, 117.0, 118.1 (2), 119.1, 120.1, 120.2, 120.7 (2), 122.0, 126.2 (2), 127.3 (2), 127.9 (2), 128.4 (2), 129.6, 131.6, 132.6, 133.5, 134.4, 137.2, 138.6, 142.9, 146.9, 165.6 ppm; MS (ESI) m/z: M+1 peak found, 538.02 (M+1 peak calculated, 538.11); Anal. Calcd. for: C, 62.56; H, 3.56; N, 13.03; S, 5.97. Found: C, 62.51; H, 3.51; N, 13.10; S, 5.87.

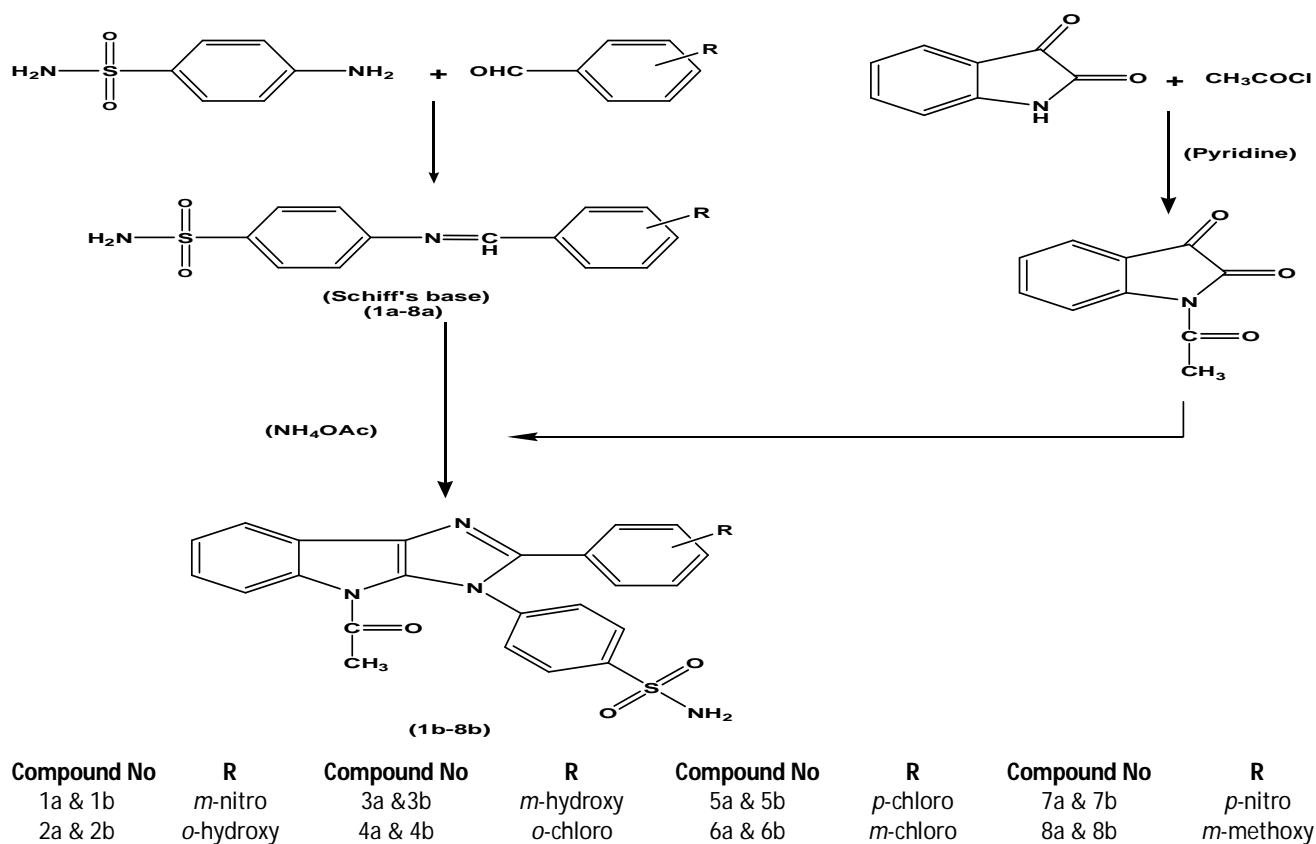
4-[2-(2'-Hydroxyphenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzenesulfonamide (10b, $\text{C}_{28}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$)

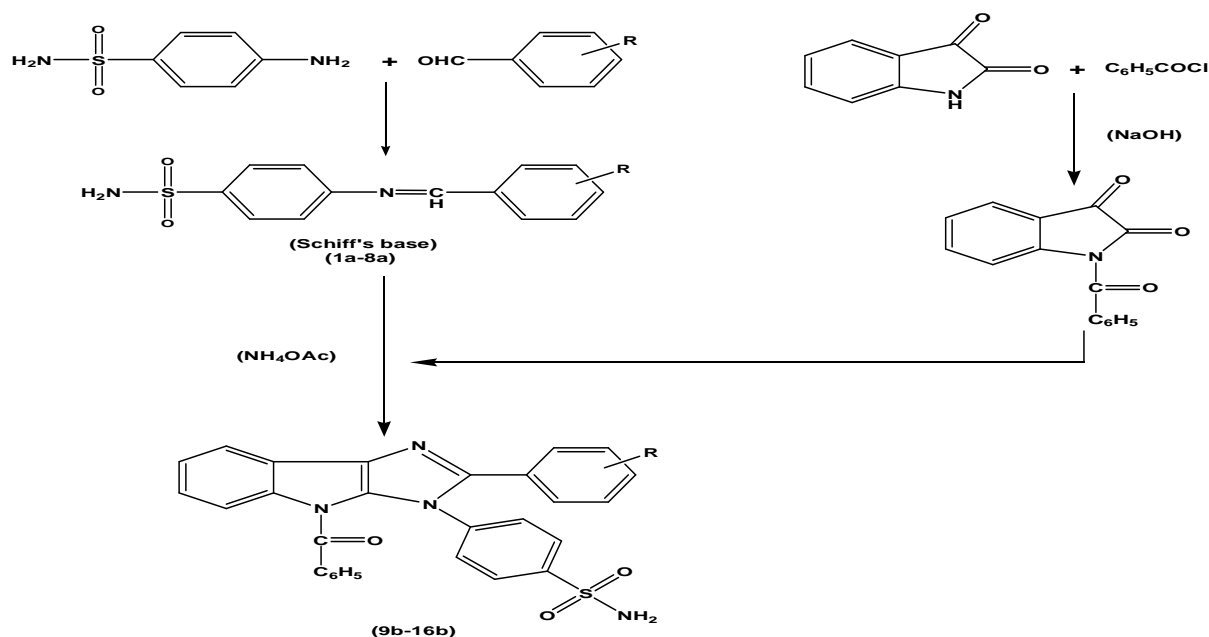
Mp (°C): 83; Rf value: 0.76; FTIR (KBr): 3570.42 (O-H), 3322.76 (N-H), 3080.17 (Ar C-H), 1672.24 (C=O), 1560.10 (C=N), 1545.22 (C=C), 1325.11 (S-N), 1195.78 (C-N), 1063.56 (S=O), 672.42 (C-S) cm^{-1} ; ^1H NMR (DMSO- d_6): δ = 6.42 (s, 2H, NH_2 , D_2O exchangeable), 6.90-7.98 (m, 17H, Ar-H), 8.07 (s, 1H, OH, D_2O exchangeable) ppm; ^{13}C NMR: δ = 110.1, 114.4, 116.5, 117.0, 118.1, 119.1 (2), 120.2, 120.7 (2), 122.0, 126.2 (2), 126.9, 127.3 (2), 127.9 (2), 128.2, 128.6, 132.6, 133.5, 134.6, 137.2, 138.6, 142.9, 153.3, 165.5 ppm; MS (ESI) m/z: M+1 peak found, 509.03 (M+1 peak calculated, 509.11); Anal. Calcd. for: C, 66.13; H, 3.96; N, 11.02; S, 6.31. Found: C, 66.08; H, 3.89; N, 10.98; S, 6.28.



Table 2: Anthelmintic Activity

Compound No.	Mean paralyzing time (min)			Mean death time (min)		
	<i>M. konkanensis</i>	<i>P. corethruses</i>	<i>Eudrilus</i>	<i>M. konkanensis</i>	<i>P. corethruses</i>	<i>Eudrilus</i>
1b	15.50 ± 0.50	16.00 ± 1.00	20.50 ± 0.50	22.33 ± 1.52	24.83 ± 1.04	28.50 ± 0.50
2b	14.16 ± 1.25	15.16 ± 1.25	20.33 ± 0.76	23.16 ± 0.76	23.00 ± 1.00	28.83 ± 0.76
3b	15.37 ± 0.57	16.33 ± 0.57	20.16 ± 0.57	24.33 ± 0.57	25.50 ± 0.50	29.16 ± 0.76
4b	26.66 ± 0.57	14.00 ± 1.00	19.50 ± 0.50	33.66 ± 0.57	23.66 ± 0.57	27.66 ± 0.57
5b	32.66 ± 0.57	22.33 ± 0.57	26.33 ± 0.57	39.16 ± 0.76	32.83 ± 0.76	39.83 ± 0.76
6b	16.83 ± 0.76	16.00 ± 1.00	20.00 ± 1.00	23.33 ± 0.57	25.00 ± 1.00	29.33 ± 0.57
7b	38.16 ± 1.75	23.33 ± 0.57	27.83 ± 0.76	45.66 ± 0.57	33.00 ± 1.00	41.33 ± 0.57
8b	15.33 ± 0.57	16.33 ± 0.57	20.33 ± 1.52	22.33 ± 0.57	24.33 ± 0.57	29.00 ± 1.00
9b	29.16 ± 1.25	27.33 ± 0.57	32.33 ± 0.57	38.66 ± 0.57	37.66 ± 0.57	42.66 ± 1.15
10b	22.00 ± 1.00	25.00 ± 1.00	29.33 ± 0.57	28.33 ± 0.57	35.33 ± 0.57	39.66 ± 1.15
11b	26.00 ± 1.00	28.83 ± 0.76	34.00 ± 1.00	32.00 ± 1.00	39.00 ± 1.00	45.66 ± 1.52
12b	27.00 ± 1.00	14.00 ± 1.00	19.00 ± 1.00	34.00 ± 1.00	23.00 ± 1.00	28.00 ± 1.00
13b	22.66 ± 1.15	25.33 ± 0.57	31.33 ± 0.57	29.00 ± 1.00	36.00 ± 1.00	44.00 ± 1.00
14b	21.66 ± 1.15	25.00 ± 1.00	29.00 ± 1.00	26.00 ± 1.00	35.00 ± 1.00	39.00 ± 1.00
15b	25.00 ± 1.00	26.00 ± 1.00	32.00 ± 1.00	31.00 ± 1.00	37.00 ± 1.00	47.00 ± 1.00
16b	26.00 ± 1.00	16.00 ± 1.00	20.33 ± 0.57	31.00 ± 1.00	24.00 ± 1.00	29.00 ± 1.00
Control	-	-	-	-	-	-
Mebendazole	18.22 ± 1.00	16.32 ± 0.64	20.83 ± 0.76	25.37 ± 1.00	23.43 ± 0.72	29.33 ± 1.52

**Scheme I**



Compound No	R	Compound No	R	Compound No	R	Compound No	R
1a & 9b	<i>m</i> -nitro	3a & 11b	<i>m</i> -hydroxy	5a & 13b	<i>p</i> -chloro	7a & 15b	<i>p</i> -nitro
2a & 10b	<i>o</i> -hydroxy	4a & 12b	<i>o</i> -chloro	6a & 14b	<i>m</i> -chloro	8a & 16b	<i>m</i> -methoxy

Scheme II

4-[2-(3'-Hydroxyphenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzene sulfonamide (11b, C₂₈H₂₀N₄O₄S)

Mp (°C): 99; Rf value: 0.66; FTIR (KBr): 3595.40 (O-H), 3310.67 (N-H), 3070.63 (Ar C-H), 1674.64 (C=O), 1589.82 (C=C), 1523.01 (C=N), 1331.68 (S-N), 1125.42 (C-N), 1046.42 (S=O), 683.64 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.30 (s, 2H, NH₂, D₂O exchangeable), 6.71-8.43 (m, 17H, Ar-H), 8.84 (s, 1H, OH, D₂O exchangeable) ppm; ¹³C NMR: δ = 110.1, 114.4, 116.5, 117.0, 118.1, 119.1, 120.2 (2), 120.7 (2), 122.0, 126.2 (2), 126.9, 127.3 (2), 127.8 (2), 128.2, 128.6, 132.6, 133.5, 134.6, 137.4, 138.4, 139.6, 157.0, 165.6 ppm; Anal. Calcd. for: C, 66.13; H, 3.96; N, 11.02; S, 6.31. Found: C, 66.09; H, 3.94; N, 11.01; S, 6.30.

4-[2-(2'-Chlorophenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzene sulfonamide (12b, C₂₈H₁₉ClN₄O₃S)

Mp (°C): 79; Rf value: 0.53; FTIR (KBr): 3320.56 (N-H), 3095.12 (Ar C-H), 1661.42 (C=O), 1592.42 (C=N), 1548.28 (C=C), 1325.11 (S-N), 1143.13 (C-N), 1049.98 (S=O), 756.08 (C-Cl), 686.68 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.72 (s, 2H, NH₂, D₂O exchangeable), 6.90-8.87 (m, 17H, Ar-H) ppm; ¹³C NMR: δ = 110.1, 117.0, 118.2 (2), 119.2, 120.1, 120.2, 120.7 (2), 122.0, 126.2 (2), 127.2 (2), 127.8 (2), 128.6 (2), 129.7, 131.4, 131.9, 132.9, 134.4, 136.2, 138.6, 142.8, 145.6, 165.7 ppm; MS (ESI) m/z: M+1 peak found, 526.99 (M+1 peak calculated, 527.09); Anal. Calcd. for: C, 63.81; H, 3.63; N, 10.63; S, 6.08. Found: C, 63.85; H, 3.59; N, 10.61; S, 6.06.

4-[2-(4'-Chlorophenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzene sulfonamide (13b, C₂₈H₁₉ClN₄O₃S)

Mp (°C): 73; Rf value: 0.49; FTIR (KBr): 3327.89 (N-H), 3052.22 (Ar C-H), 1668.24 (C=O), 1548.22 (C=C), 1535.12

(C=N), 1325.11 (S-N), 1193.12 (C-N), 1068.72 (S=O), 763.24 (C-Cl), 681.12 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.51 (s, 2H, NH₂, D₂O exchangeable), 6.62-8.88 (m, 17H, Ar-H) ppm; ¹³C NMR: δ = 110.1, 117.0, 118.2 (2), 119.1, 120.2, 120.4, 120.8 (2), 122.0, 126.2 (2), 127.2 (2), 127.8 (2), 128.6 (2), 129.6, 131.5, 132.6, 132.9 (2), 134.4, 136.3, 138.6, 142.7, 165.5 ppm; Anal. Calcd. for: C, 63.81; H, 3.63; N, 10.63; S, 6.08. Found: C, 63.78; H, 3.61; N, 10.60; S, 6.10.

4-[2-(3'-Chlorophenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzene sulfonamide (14b, C₂₈H₁₉ClN₄O₃S)

Mp (°C): 66; Rf value: 0.72; FTIR (KBr): 3340.56 (N-H), 3075.12 (Ar C-H), 1655.24 (C=O), 1542.22 (C=C), 1515.11 (C=N), 1325.08 (S-N), 1143.13 (C-N), 1039.42 (S=O), 763.68 (C-Cl), 684.12 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.51 (s, 2H, NH₂, D₂O exchangeable), 6.62-8.88 (m, 17H, Ar-H) ppm; ¹³C NMR: δ = 110.2, 117.0, 118.1 (2), 119.2, 120.1, 120.2, 120.7 (2), 122.0, 126.2 (2), 127.3 (2), 127.9 (2), 128.4 (2), 129.5, 131.6 (2), 132.6, 133.5, 134.5, 137.2, 138.1, 142.6, 165.5 ppm; Anal. Calcd. for: C, 63.81; H, 3.63; N, 10.63; S, 6.08. Found: C, 63.88; H, 3.60; N, 10.59; S, 6.09.

4-[2-(4'-Nitrophenyl)imidazo[4,5-*b*]N-benzoylindole-3(4*H*)-yl]benzenesulfonamide (15b, C₂₈H₁₉N₅O₅S)

Mp (°C): 104; Rf value: 0.65; FTIR (KBr): 3337.89 (N-H), 3096.12 (Ar C-H), 1659.24 (C=O), 1598.12 (C=N), 1541.22 (C=C), 1399.98 (NO₂), 1325.45 (S-N), 1094.49 (C-N), 1046.16 (S=O), 853.12 (C-N stretching for NO₂), 688.84 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.41 (s, 2H, NH₂, D₂O exchangeable), 6.62-8.81 (m, 17H, Ar-H) ppm; ¹³C NMR: δ = 110.2, 117.0, 118.1 (2), 119.2, 120.1, 120.2, 120.7, 122.0, 126.2 (2), 127.3 (2), 127.9 (2), 128.4 (2), 129.5,



131.6 (2), 132.6, 133.5, 134.5, 137.2, 138.1, 142.6, 144.2, 165.5 ppm; Anal. Calcd. for: C, 62.56; H, 3.56; N, 13.03; S, 5.97. Found: C, 62.51; H, 3.52; N, 12.98; S, 5.90.

4-[2-(3'-Methoxyphenyl)imidazo[4,5-b]N-benzoylindole-3(4H-y)]benzene sulfonamide (16b, C₂₉H₂₂N₄O₄S)

Mp (°C): 88; Rf value: 0.36; FTIR (KBr): 3329.13 (N-H), 3012.12 (Ar C-H), 1678.24 (C=O), 1527.22 (C=C), 1517.11 (C=N), 1343.11 (S-N), 1149.50 (C-O-C), 1193.13 (C-N), 1062.16 (S=O), 682.98 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 3.31 (s, 3H, OCH₃), 6.30 (s, 2H, NH₂, D₂O exchangeable), 6.61-8.43 (m, 17H, Ar-H) ppm; ¹³C NMR: δ = 53.8, 110.2, 117.0, 118.2 (2), 119.2, 120.1, 120.2, 120.7, 122.0, 126.1 (2), 127.4 (2), 127.8 (2), 128.4 (2), 129.5, 131.6 (2), 132.6, 133.5, 134.5, 137.2, 138.2, 142.6, 159.2, 165.5 ppm; MS (ESI) m/z: M+1 peak found, 523.09 (M+1 peak calculated, 523.14); Anal. Calcd. for: C, 66.65; H, 4.24; N, 10.73; S, 6.14. Found: C, 66.61; H, 4.20; N, 10.69; S, 6.16.

RESULTS AND DISCUSSION

Structures of all the newly synthesized compounds were confirmed by FTIR, ¹H NMR and Mass spectral analysis. The IR spectra of the newly synthesized compounds showed the presence of characteristic absorption bands in the region 3310-3350 cm⁻¹ for N-H in NH₂, 3012-3096 cm⁻¹ for aromatic C-H stretching, 1650-1680 cm⁻¹ for C=O stretching, 1500-1600 cm⁻¹ for C=N stretching, 750-600 cm⁻¹ for C-S stretching respectively. ¹H NMR spectra of synthesized compounds showed the characteristic peaks in the region 5.02-6.54 ppm for -NH₂ proton and 6.67-7.80 ppm for aromatic protons.

The synthesized compounds were screened for *in vitro* anticancer activity using HEP-2 cell line. Compounds 1b, 2b, 7b, 10b and 16b showed significant anticancer activity at CTC₅₀ values 46.25µg/ml, 32.41µg/ml, 38.41µg/ml, 43.25µg/ml and 34.46µg/ml respectively under *in vitro* anticancer screening using HEP-2 cell line by SRB assay in comparison to standard 5-fluorouracil showing CTC₅₀ value at 31.76µg/ml. Compounds 3b, 8b, 11b and 14b showed moderate anticancer activity at CTC₅₀ values 86.32µg/ml, 82.33µg/ml, 74.22µg/ml and 69.13µg/ml respectively under *in vitro* anticancer screening using HEP-2 cell line by SRB assay in comparison to standard. Presence of phenolic group in compound 2b and 10b significantly affect activity due to the binding capability to the cytoplasmic hormone receptors. Compound 1b and 7b containing nitro group which is electronegative in nature, make the compound potent. Presence of methoxy group also increases the potential of compound 16b.

Anthelmintic activity of the synthesized novel imidazoles was carried out against three different species of earthworms *M. konkanensis*, *P. corethruses* and *Eudrilus* at 4mg/ml concentration. All the novel imidazoles showed moderate to good activity at 100mg in tween 80 (0.5%) and distilled water. Comparison of Anthelmintic data (Table 2) revealed that derivative 1b, 2b, 3b, 6b and 8b possessed higher activity against *M. konkanensis* species in comparison to standard Mebendazole. 1b, 2b, 3b, 6b,

8b, 12b and 16b possessed higher activity against *P. corethruses* species in comparison to standard Mebendazole. 1b, 2b, 3b, 4b, 6b, 8b, 12b and 16b possessed higher activity against *Eudrilus* species in comparison to standard Mebendazole.

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