Synthesis, reactions and anthelmintic activity of 1-[benzimidazol-2-yl]-4-formyl-3-[2′(-substituted phenyl) indole-3-yl] pyrazoles

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3-Acetyindolbenzimidazol-2-yl hydrazones 3, which in turn are prepared by the reaction of 2-hydrazino benzimidazoles 1 and 3-acetyl indoles 2 in ionic liquid [bmim]PF₆ upon treatment with Vilsmeier Haack reagent undergo cyclization to give the 1-[benzimidazol-2-yl]-4-formyl-3-[2′(-substituted phenyl) indol-3-yl] pyrazoles 4, which on further treatment with substituted acetophenones and substituted anilines in ionic liquid [bmim]PF₆/ethanol-NaOH/ethanol-AcOH afford the corresponding 1-[benzimidazol-2-yl]-3-[2′(-substituted phenyl) indol-3-yl]-4-[1′-substituted phenyl prop-2′-ene-1′-ones]-yl pyrazoles 5 and 1-[benzimidazol-2-yl]-3-[2′-(substituted phenyl) indol-3-yl]-4-[N-methylene anilines]-yl pyrazoles 6. The synthesized compounds have been characterized by analytical and spectral (IR, ¹H and ¹³C NMR and FAB mass) data and found to display promising activity when screened for anthelmintic activity against *Pheritima posthuma*.

Keywords: 3-Acetyindolbenzimidazol-2-yl hydrazones, 1-[benzimidazol-2-yl]-4-formyl-3-[2′(-substituted phenyl) indol-3-yl] pyrazoles, 1-[benzimidazol-2-yl]-3-[2′-(substituted phenyl) indol-3-yl]-4-[1′-substituted phenyl prop-2′-ene-1′-ones]-yl pyrazoles, 1-[benzimidazol-2-yl]-3-[2′-(substituted phenyl) indol-3-yl]-4-[N-methylene anilines]-yl pyrazoles, ionic liquid, anthelmintic activity

Heterocyclic nitrogenous compounds and their fused analogs represent an important class of heterocyclic compounds. They exist in numerous natural products and display a wide range of biological and pharmaceutical activities. Benzimidazoles have been exploited over the past few decades because of the wide range of activities displayed by this class of heterocycles. The indoles are also found to be associated with anticonvulsant and antioxidant activities. Pyrazoles show antifungal, anticancer and antioxidant activities, and act as hepatoselective HMG-CoA reductase. In the course of synthesis of bioactive benzimidazoles some novel polycyclic benzimidazoles have been prepared incorporating indoles and pyrazoles to study the additive effect of these to enhance the biological activity.

In the past decade ionic liquids have developed from a curiosity to a new class of solvent with attractive properties, like higher solubility of solutes as compared to classical organic solvents, no effective vapour pressure, high stability over a wide range of temperatures, easy recyclability and reusability which made the synthesis reasonable, inexpensive and environmentally benign.

In view of this, reaction between 2-hydrazinobenzimidazole and 3-acetyl indoles were carried out for the first time in ionic liquid [bmim]PF₆ leading to the formation of 3-acetyl indol benzimidazol-2-yl hydrazones. The same can also be prepared by conventional method by refluxing in ethanol for a long time (5-6 hr) as compared to ionic liquid method having reaction time of only 30 min.

Further, these hydrazones were subjected to Vilsmeier Haack reaction *i.e.* treated with DMF-POCI₃ to give 1-[benzimidazol-2-yl]-4-formyl-3-[2′-substituted phenyl) indol-3-yl] pyrazoles 4.

The formyl group is very reactive and treatment with substituted acetophenones and substituted anilines in [bmim]PF₆ / ethanol-NaOH / ethanol-AcOH gave corresponding 1-[benzimidazol-2-yl]-3-[2′-(substituted phenyl)indol-3-yl]-4-[1′-substituted phenyl prop-2′-ene-1′-ones]-yl pyrazoles 5 and 1-[benzimidazol-2-yl]-3-[2′-(substituted phenyl) indol-3-yl]-4-[N-methyleneanilines]-yl pyrazoles 6 (*Scheme I*). The synthesized compounds exhibited promising anthelmintic activity.
Scheme I
Results and Discussion

Ionic liquid is a better reaction medium than other organic solvents. Therefore, reaction was carried out in versatile green solvent [bmmim]PF$_6$ and recycled indefinitely for further synthesis of other compounds. The reaction was also carried out by conventional methods using ethanol as a solvent which required longer reaction time than in ionic liquid.

Formation of hydrazone 3 was confirmed by IR spectra in which it shows bands due to >NHN= at 3400-3300 cm$^{-1}$ besides bands at 3200 and 3100 cm$^{-1}$ for >NH of benzimidazole and >NH of indole. A band around 1605-1620 confirms the presence of –NHN=C bond. $^1$H NMR shows peaks at δ 10-11 for >N=NC=C and 1.75-2.00 for =C- methyl group. $^{13}$C NMR shows peak at δ 164.4 and 28.5 for C=N and =C- methyl group respectively. Further, mass spectrum of 3a shows M$^+$ at m/z 365.

Formation of pyrazole derivative 4 from hydrazone 3 was confirmed by IR spectra which shows a band at 1720-1740 cm$^{-1}$ for –CHO group attached at 4-position of pyrazole ring and disappearance of peak due to >NHN= and >C=N. $^1$H NMR shows singlets at δ 10-10.5 and 7.81 for CHO and =CH of pyrazole ring respectively with disappearance of peak due to >NHN= and =C- methyl group. $^{13}$C NMR shows peak at δ 192 for CHO and δ 149 and 145 for two pyrazolyl carbons. Further, mass spectrum shows M$^+$ at m/z 402 (4a).

Formation of chalcones 5 was confirmed by IR spectra in which it shows bands at 1660-1680 cm$^{-1}$ due to α,β-unsaturated ketone. $^1$H NMR shows disappearance of singlet peak due to –CHO at δ 7.81 and appearance of doublets at δ 6.72 and 7.22 due to CO–CH=CH and COCH=CH$^–$. Further, mass spectrum shows M$^+$ at m/z 504 (5a). Formation of Schiff bases 6 were confirmed by IR spectra in which it shows bands at 1568-1620 cm$^{-1}$ due to >C=N. $^1$H NMR shows peak at δ 8.02 due to N=CH. $^{13}$C NMR shows peak due to CH=N at δ 164.8. Further, mass spectrum shows M$^+$ at m/z 477 (6a). Characterization and spectral data are recorded in Tables I and II.

Anthelmintic Activity

Helminth is a general term meaning worm. The most common worm is the earth worm a member of phylum Annelida.

Indian adult earth worm (Pheretima posthuma) collected from moist soil and washed with normal saline to remove all faecal matter were used for anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in breadth were used for all experimental protocols due to their anatomical and physiological resemblance with the intestinal round worm parasites of human being$^{13,14}$. All the synthesized heterocyclic derivatives were dissolved in minimum quantity of DMF (1% and 2% solutions were made) and 2 mL solution was taken in a petri-dish. The volume was adjusted to 10 mL with normal saline water. All drugs and compounds solutions were freshly prepared before starting the experiment. Groups of six earthworms were released into 10 mL of desired formulation as followed: Synthesized derivatives (2 mg/mL) and Albendazole (Bandy, Mankind Pharma Ltd., New Delhi) 20 mg/mL in normal saline containing 5% DMF. Observations were made for the time taken for paralysis and death of individual earthworms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour$^{15-17}$. Death of motion-less earthworm was also ascertained by placing the earthworm in lukewarm water, which stimulates movement if the worm is alive. Experiments were carried out in duplicate and average values were recorded.

The time taken by the earthworms to become motionless was noted as paralytic time (PT). The time of death was noted as lethal time (LT). The result shows that paralytic time was 3-6 min and lethal time was 4-8 min for compounds 3-6 at 1% concentration. With 2% concentration, the PT and LT was 2-5 min and 3-6 min respectively. The standard drug Albendazole shows the paralytic time 5 min and lethal time 7 min with 1% concentration. With 2% concentration the PT and LT was 4 min and 6 min respectively. It may be concluded that these benzimidazole derivatives 3-6 are very active. Some of the compounds are even more active than the standard drug. This may be attributed to chloro and fluoro substitution in the ring. The results are recorded in Table III.

Experimental Section

Melting points are uncorrected and were obtained in open glass capillaries using Gallenkamp melting point apparatus. The IR spectra were recorded on a 800S Shimadzu IR spectrometer in KBr pellets and band positions are reported in wave numbers (cm$^{-1}$). The $^1$H NMR spectra and $^{13}$C NMR spectra have been recorded on Jeol 300 MHz instrument in DMSO-
Chemical shifts (δ) are given in ppm. The mass spectra were recorded on Jeol SX 102 (FAB) mass spectrometer. Elemental analysis was performed at Central Drug Research Institute, Lucknow, India.

**General procedure for the synthesis of 3-acetyl indol benzimidazol-2-yl-hydrazones, 3**

**A. Conventional method**

A mixture of 2-hydrazinobenzimidazole 1 (0.01 mol) and 3-acetyl indole (0.01 mol) in ethanol (10 mL) and a few drops of glacial acetic acid were heated on a water bath for 5-6 hr. The mixture was cooled, filtered and product washed with dil HCl and cold ethanol. It was purified by recrystallization from ethanol to give 3 in 60-65% yield. The analytical and spectral characterization data of compounds synthesized are recorded in Tables I and II respectively.

**B. Ionic liquid mediated synthesis of 3**

A mixture of 2-hydrazinobenzimidazole (0.003 mol), 3-acetylindole (0.003) and ionic liquid [bmim]PF₆ (5 mL) were taken in a round bottom flask. It was heated at 60-70°C for 30 min. The progress of reaction was monitored by TLC. After completion of the reaction the contents were neutralized by 10% aqueous sodium bicarbonate solution and extraction was carried out with ethylacetate (3×10 mL). The solvent was removed under reduced pressure. The pasty mass thus obtained was extracted with diethyl ether (3 × 10 mL), dried over anhydrous sodium sulphate and the ether distilled off. The product so obtained was purified by recrystallization with ethanol / column chromatography to give 3 in 90-95% yield. The ionic liquid layer was washed with water (3 × 5 mL) and kept for 2 hr at 80-85°C at reduced pressure. This ionic liquid was reused for synthesis of other compounds and recycled indefinitely. The characterization and analytical data are the same as prepared by conventional method.

Vilsmeier Haack reaction for the synthesis of 1-[benzimidazol-2-yl]-4-formyl indole-3-yl pyrazoles, 4

Compound 3 (0.05 mol) was dissolved in Vilsmeier Haack reagent (6 mL DMF and 1.5 mL POCl₃) and
Table II — Spectral characterization data of compounds 3 and 4

<table>
<thead>
<tr>
<th>Compd</th>
<th>$^1$H NMR (δ, ppm)</th>
<th>$^{13}$C NMR (δ, ppm)</th>
<th>MS: M$^+$ (m/z)</th>
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<tr>
<td>3a</td>
<td>11.61 (s, 1H, &gt;NHN=), 9.52 (s, 1H, &gt;NH)</td>
<td>164.4 (NHN=)</td>
<td>365</td>
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<td>9.02 (s, 1H, &gt;NH), 6.85-7.56 (m, 13H, Ar-H)</td>
<td>157.8 (N=C(NH)$_2$)</td>
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<td>1.75 (s, 3H, CH$_3$)</td>
<td>28.4 (=C–CH$_3$), 137.9, 135.5, 128.7, 127.9, 127.6, 126, 124.1, 122.9, 121.7, 120.5, 119.6, 115.4, 111, 108.6 (Ar-H)</td>
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<tr>
<td>3b</td>
<td>10.50 (s, 1H, &gt;NHN=), 9.62 (s, 1H, &gt;NH)</td>
<td>164.8 (NHN=), 157.8 (N=C(NH)$_2$)</td>
<td>417.5</td>
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<td>9.23 (s, 1H, &gt;NH), 6.82-7.59 (m, 11H, Ar-H)</td>
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<td>1.82 (s, 3H, CH$_3$)</td>
<td>28.9 (=C–CH$_3$), 137.6, 135.4, 128.4, 127.6, 127.8, 126, 124.5, 122.8, 121.4, 120.4, 119.5, 115.2, 111.01, 108.5 (Ar-H)</td>
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<td>3c</td>
<td>10.81 (s, 1H, &gt;NHN=), 9.74 (s, 1H, &gt;NH)</td>
<td>164.6 (NHN=), 158.0 (N=C(NH)$_2$)</td>
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<td>9.45 (s, 1H, &gt;NH), 6.86-7.91 (m, 12H, Ar-H)</td>
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<td>1.86 (s, 3H, CH$_3$)</td>
<td>135.4, 128.6, 127.8, 27.5, 126, 124.2, 123, 121.8, 120.6, 120.1, 115.8, 111.1, 109.0 (Ar-H)</td>
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<td>3d</td>
<td>10.85 (s, 1H, &gt;NHN=), 9.82 (s, 1H, &gt;NH)</td>
<td>165.0 (NHN=), 158.0 (N=C(NH)$_2$), 28.2</td>
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<td>9.25 (s, 1H, &gt;NH), 6.25-7.68 (m, 12H, Ar-H)</td>
<td>157.8, 135.6, 128.5, 127.8, 127.6, 126, 124.5, 122.8, 121.4, 120.4, 119.5, 115.2, 111.01, 108.5 (Ar-H)</td>
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<td>2.41 (s, 3H, –C$_6$H$_4$–CH$_3$), 1.79 (s, 3H, =C–CH$_3$)</td>
<td>124.1, 124, 122.8, 121.6, 120.8, 119.5, 115.8, 111.2, 108.7 (Ar-H)</td>
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<td>3e</td>
<td>11.23 (s, 1H, &gt;NHN=), 9.01 (s, 1H, &gt;NH)</td>
<td>164.9 (NHN=), 157.8 (N=C(NH)$_2$), 28.5</td>
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<td>6.52-7.63 (m, 13H, Ar-H), 3.98 (s, 3H, N-CH$_3$)</td>
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<td>3f</td>
<td>11.02 (s, 1H, &gt;NHN=), 9.69 (s, 1H, &gt;NH)</td>
<td>165.0 (NHN=), 158.0 (N=C(NH)$_2$), 29.8</td>
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<td>2.35 (s, 3H, –C$_6$H$_4$–CH$_3$), 1.78 (s, 3H, =C–CH$_3$)</td>
<td>137.8, 135.6, 128.5, 127.8, 127.6, 126, 124.5, 122.8, 121.6, 120.8, 119.5, 115.8, 111.2, 108.7 (Ar-H)</td>
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<td>3g</td>
<td>11.08 (s, 1H, &gt;NHN=), 6.58-7.68 (m, 13H, Ar-H)</td>
<td>165.01 (NHN=), 157.9 (N=C(NH)$_2$), 35.9 (NCH$_3$), 34.0</td>
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<td>3.98 (&gt;NCH$_3$), 3.15 (s, 3H, &gt;NCH$_3$), 1.69 (s, 3H, =C–CH$_3$)</td>
<td>(NCH$_3$), 137.8, 135.4, 128.7, 127.7, 127.5, 126, 124.2, 121.5, 120.6, 118.5, 115.1, 110, 107.5 (Ar-H)</td>
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<tr>
<td>4a</td>
<td>10.21 (s, 1H, –CHO), 9.53 (s, 1H, &gt;NH)</td>
<td>192 (CHO), 149 (C-3), 145 (C-5), 136.4 (C-4), 137.9, 135.2, 128.5, 127.8, 127.6, 126, 124.2, 122.8, 121.5, 120.6, 118.5, 115.1, 110, 107.5 (Ar-H)</td>
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<td>8.82 (s, 1H, &gt;NH), 7.83 (s, 1H, =CH)</td>
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<td>6.51-7.63 (m, 13H, Ar-H)</td>
<td>128.6, 127.9, 127.6, 126, 124.3, 122.9, 127.6, 126, 124.3, 122.9, 121.6, 120.4, 118.6, 115.2, 110.2, 108.5 (Ar-H)</td>
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<tr>
<td>4b</td>
<td>10.15 (s, 1H, –CHO), 9.62 (s, 1H, &gt;NH)</td>
<td>(CHO), 149 (C-3), 145 (C-5), 136 (C-4), 151.4</td>
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tirred at RT for 4-5 hr. Then the contents were poured over crushed ice (previously neutralized with NaHCO₃), solid separated out, which was filtered, washed with water, dried and purified by recrystallization from ethanol to give 4 in 80-86% yield. The analytical and spectral data of compounds synthesized are recorded in Tables I and II.

Synthesis of 1-[benzimidazol-2-yl]-3-[2′-phenylindol-3-yl]-4-[1′-phenyl prop-2′-ene-1′-one]-yl pyrazole, 5a

**A. Conventional method**

A mixture of 4a (0.001 mol) and acetophenone (0.001 mol) in ethanol (50 mL) was cooled to 5-10°C. Sodium hydroxide (70%, 5 mL) was added to it drop wise with constant stirring. The reaction mixture was stirred for 2 hr and left overnight and then neutralized with concentration hydrochloric acid. The solid which separated out was filtered and purified by recrystallization from pet ether ethylacetate (20 : 80). Yield 65%; m.p. 205°C. Anal. Calcd for C₃₃H₂₂N₅O: C, 78.57; H, 4.36; N, 13.88. Found: C, 78.53; H, 4.40; N, 13.84%. IR (KBr): 3320 (>NH), 3090 (>NH), 1660 (>C=O), 1450 (C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 6.70 (d, 1H, COC₆H₅=CH), 7.23 (d, 1H, CH=C₆H₅), 7.50-8.09 (m, 13H, Ar-H), 8.86 (s, 1H, >NH), 9.58 (s, 1H, >NH); MS: m/z 437.5

1-[Benzimidazol-2-yl]-3-[2′-phenylindol-3-yl]-4-[1′-p-fluorophenyl-prop-2′-ene-1′-one]-yl pyrazole, 5b

Yield 68%; m.p. 198°C. Anal. Calcd for C₃₃H₂₁F₅N₅O: C, 75.86; H, 4.02; N, 17.40. Found: C,
Compounds have the same analytical and spectral characteristics as prepared by conventional method.

**B. Ionic liquid mediated synthesis of 5**

A mixture of 4a (0.001 mol) and acetophenone/ p-fluoroacetophenone (0.001 mol) in [bmim]PF$_6$ (5 mL) was heated to 60-70°C for 30-40 min. The progress of reaction was monitored by TLC. Work-up method was the same as the one used for compounds 3. Yield, 5a 90%; 5b 92%.

Compounds have the same analytical and spectral characteristics as prepared by conventional method.

1-[Benzimidazol-2-yl]-3-[2'-phenyl indol-3-yl]-4-[N- methylene aniline]-yl-pyrazole, 6a

A mixture of 4a (0.001 mol), aniline (0.01 mol) and acetic acid (0.5 mL) in methanol (10 mL) was refluxed for 4 hr. The product was then isolated by filtration, washed with water and purified by recrystallization from ethanol. Yield 66%; m.p.195°C. Anal. Calcd for C$_{31}$H$_{23}$N$_6$: C, 77.98; H, 4.40; N, 17.61. Found C, 78.02; H, 4.42; N, 17.59%. IR (KBr): 3360 (>NH), 3100 (>NH), 1568 (C=N), 1436 (C=C) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 6.58-7.83 (m, 13H, Ar-H), 148 (C-3), 143 (C-5), 136 (C-4), 135-108 (Ar-H); MS: m/z 759.18; H, 4.06; N, 16.93%. IR (KBr): 3350 (>NH), 1680 (>C=O), 1460 (C=C) cm$^{-1}$;

**References**