Chapter 5

Synthesis and Biological Evaluation of Some Pyrazolylbenzimidazoles
CHAPTER 5. Synthesis and Biological Evaluation of Some Pyrazolylbenzimidazoles

5.1. Motivation for the current work

Over the years, azoles constitute immensely important members of the biologically active aromatic heterocycles family due to their presence in numerous bioactive natural products as privileged pharmacophores.\(^1\) Recently, imidazole pharmacophore has been extensively studied as an important scaffold for the development of novel anticancer, anti-inflammatory (AI), antimicrobial, antiulcer etc agents.\(^2\,^3\) Dacarbazine (1)\(^4\) and azathioprine (2)\(^5\) are clinically used anticancer drugs bearing imidazole ring in their structure.

Benzimidazoles are aromatic heterocyclic compounds which share a fundamental structural characteristic of six-membered benzene fused to five-membered imidazole. This basic ‘6+5’ heterocyclic structure is similar to another class of important chemical compounds, the purine nucleobases such as adenine and guanine, as well as uric acid, and caffeine.\(^6\) Due to this fundamental structural similarity, it is quite understandable that benzimidazole derivatives have been found to be biologically active molecules, such as vitamin B\(_{12}\) and a variety of antimicrobial, antiparasitic and antitumor agents.\(^7\,^8\,^9\) Apart from their place in biomedical research, benzimidazoles also have a prominent place in organocatalysis, organometallic,\(^10\,^11\) and materials\(^12\) chemistry for two main reasons stemming from their molecular architecture. Firstly, imidazole is a precursor to \(N\)-heterocyclic carbenes and secondly, the benzene ring provides a convenient scaffold to which additional functionality may be easily added to modify the spatial and electronic characteristics of a benzimidazole derivative. This combination of a reactive carbene centre with a modifiable backbone is without doubt one of the reasons for the recent rise in study and use of benzimidazoles and their \(N\)-heterocyclic carbene derivatives.
In addition to benzimidazoles, pyrazoles are also known to exhibit various biological activities such as, anticancer, anti-inflammatory, antimicrobial etc. Literature survey indicates that the presence of two pharmacophores in a single heterocyclic entity may be crucial in enhancing biological activity. Keeping above observations in mind coupled with our ongoing research work on biologically active heterocycles, we were prompted to design and synthesize a series of pyrazolylbenzo[d]imidazoles for evaluation for their in vivo anti-inflammatory and in vitro antimicrobial activity. Efforts are underway to get these compounds screened for their anticancer activity.

### 5.2. Synthetic discussion of pyrazolylbenzimidazoles (12 and 13)

Synthesis of target pyrazolylbenzo[d]imidazoles (12 and 13) have been accomplished by the condensation of appropriate 4-formylpyrazoles (8 and 10) with ortho-phenylenediamine (11) in refluxing DMF/H$_2$O using oxone as oxidant as depicted in the Scheme 5.1. The 4-formylpyrazoles were in turn synthesized from

![Scheme 5.1. Synthesis of pyrazolylbenzo[d]imidazoles (12 and 13): (i) Ethanol-water, CH$_3$COOH (catalytic), reflux, 30 min; (ii) POCl$_3$/DMF, 50-60 °C, 5-6h; (iii) NaOH/MeOH/THF overnight; (iv) oxone/DMF/H$_2$O, reflux 2-3h.](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R$_1$</th>
<th>H</th>
<th>CH$_3$</th>
<th>OCH$_3$</th>
<th>F</th>
<th>Cl</th>
<th>Br</th>
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</thead>
<tbody>
<tr>
<td>5 or 6.8.12</td>
<td>(R = H)</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>f</td>
</tr>
<tr>
<td>7, 10, 13</td>
<td>(R = SO$_2$NH$_2$)</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>f</td>
</tr>
<tr>
<td>9</td>
<td>(R = SO$_2$N=CHN(CH$_3$)$_2$)</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>f</td>
</tr>
</tbody>
</table>
corresponding hydrazones (6 and 7) under Vilsmeier Haack reaction\textsuperscript{23} conditions. In case of hydrazones 7 4-aminosulfonyl group present at \textit{para} position of aromatic ring attached to N-1 of pyrazole ring also got protected under Vilsmeier Haack conditions to afford intermediate 9 which was deprotected under basic conditions in THF-MeOH to yield 4-formylpyrazoles 10 having free 4-aminosulfonyl group. Hydrazones 6 and 7 were prepared by condensation of substituted acetophenones 5 with appropriate hydrazines 3 or 4 in refluxing ethanol-water in acidic medium.

5.2.1. Synthesis of 4-formylpyrazoles (8 and 10)

4-Formylpyrazoles 8 and 10 were synthesized from corresponding hydrazones 6 and 7 under Vilsmeier Haack conditions. A detailed description of the procedure followed for the synthesis of 4-formylpyrazoles 8 is given in chapter 2 while formation of intermediate 4-formylpyrazoles 9 having protected 4-aminosulfonyl group and its deprotection under basic conditions into free 4-aminosulfonyl group to yield compound 10 is described in detail in chapter 4.

5.2.2. Synthesis of pyrazolylbenzo[d]imidazoles (12 and 13)

The synthesis of target compounds pyrazolylbenzo[d]imidazoles 12 and 13 has been accomplished by the reaction of \textit{ortho}-phenylenediamine (11) with 4-formylpyrazoles (8 and 10) in refluxing DMF : H\textsubscript{2}O (20 : 1) in the presence of oxone. It is pertinent to mention here that the reaction does not complete without the presence of little amount of water. The target compounds were characterized by rigorous analysis of their IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and mass spectral data. The IR spectrum of 12a exhibited two absorption bands at 1597 cm\textsuperscript{-1} and 1504 cm\textsuperscript{-1} which could be attributed to C=\textit{N} stretching and N-H bending respectively. IR spectra of compounds 12b-f and 13a-f also displayed similar spectral characteristics. In case of IR spectra of 13a-f additional absorption bands appeared at 1366-1335 cm\textsuperscript{-1} and 1157 cm\textsuperscript{-1}, which were assigned to the SO\textsubscript{2} stretching. \textsuperscript{1}H NMR spectra of 12 and 13 exhibited a characteristic singlet at around δ 9.05-9.30 for C\textsubscript{5}-H of pyrazole ring. A doublet of doublet (J = 5.7-6.3 Hz and 3.0-3.3 Hz) at around δ 7.60-7.70 for two protons could be assigned to C\textsubscript{4}-H and C\textsubscript{7}-H of benzimidazole ring while presence of two protons, C\textsubscript{5}-H and C\textsubscript{6}-H, of benzimidazole ring could be ascertained by the presence of another doublet of doublet at around δ 7.22-7.25 (J = 5.7-6.0 Hz and 3.0-3.3 Hz). Presence of
methyl group at para position of aromatic ring attached to C-3 of pyrazole ring could be ascertained by a singlet for three protons at δ 2.34 in 1H NMR spectra of 12b and 13b which was further confirmed by a signal at δ 21.3 in their 13C NMR spectra. In 1H NMR spectra of 12c and 13c, a singlet for three protons at δ 3.80 could ascertain the presence of methoxy group at para position of aromatic ring attached to C-3 of pyrazole ring which was further supported by signals at δ 55.5 and δ 55.6 in their 13C NMR spectra. In case of 12d presence of fluorine at para position of aromatic ring attached to C-3 of pyrazole ring could be ascertained by three doublets at δ 162.9 (1J_CF = 246.9 Hz), 130.8 (d, 3J_CF = 8.3 Hz) and 116.1 (d, 2J_CF = 21.1 Hz) while in case of 13d three doublets appeared at 162.9 (1J_CF = 246.9 Hz), 130.9 (1J_CF = 8.3 Hz) and 115.6 (2J_CF = 21.9 Hz) in their 13C NMR spectra. The purposed structures for the target pyrazolylbenzo[d]imidazoles elucidated from their IR, 1H NMR and 13C NMR spectral analysis were further supported by their mass spectra. Presence of chlorine in 12e and 13e, and presence of bromine in 12f and 13f could be ascertained from isotopic peaks in 3:1 and 1:1 intensity ratio respectively in their mass spectra.

5.3. Biological testing results

5.3.1. Anti-inflammatory assay

The AI activity of pyrazolylbenzo[d]imidazoles was determined by carrageenan induced paw edema assay according to the protocol of Winter et al. In this method, a small amount of solution or suspension of a phlogistic agent (edemogen) is injected into the subplanter tissues of the hind paw of a rat and the amount of swelling is measured by determining the paw volume using a plethysmometer (model 7140, Ugo Basile, Italy).

5.3.1.1. Pharmacological assay

The detailed procedure of the pharmacological assay is given in the chapter 2. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follow:

% inhibition = \left( \frac{(V_t - V_0)_{control} - (V_t - V_0)_{test}}{(V_t - V_0)_{control}} \right) \times 100

Where \( V_t \) = volume of edema at specific time interval and
5.3.2. Antimicrobial assay

All the newly synthesized compounds have been evaluated for their in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria. In addition to this, these compounds were also evaluated for their in vitro antifungal activity against two fungi. Microbial strains used in the present study were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

5.3.2.1. Test microorganisms

Four bacterial and two fungal strains were selected on the basis of their clinical importance in causing diseases in humans. *Staphylococcus aureus* (*S. aureus*) (MTCC 96) and *Bacillus subtilis* (*B. subtilis*) (MTCC 121) representing Gram-positive bacteria; *Escherichia coli* (*E. coli*) (MTCC 1652) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC 741) representing Gram-negative bacteria; and two yeasts, *Candida albicans* (*C. albicans*) (MTCC 227) and *Saccharomyces cerevisiae* (*S. cerevisiae*) (MTCC 170) were used for the evaluation of antimicrobial activity of the compounds. The bacteria were subcultured on nutrient agar whereas yeast on malt yeast agar.

5.3.2.2. Antimicrobial activity

The antimicrobial activity of twelve newly synthesized compounds 12 and 13 was evaluated by the agar well diffusion method. The detailed description of procedure followed is given in chapter 2.

5.3.2.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of various compounds against bacterial strains was tested through a modified agar well diffusion method described in detail in chapter 2.

5.3.3. Biological results and discussion

5.3.3.1 Anti-inflammatory activity

All the newly synthesized pyrazolylbenzo[d]imidazoles were evaluated for their in vivo AI activity by carrageenan induced paw edema method. Increase in the
volume of paw and percentage of edema inhibition for each rat group for different compounds is summarized in Table 5.

Table 5.2. *In vivo* AI activity of pyrazolylbenzo[d]imidazoles 12 and 13

<table>
<thead>
<tr>
<th>Compound</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.11±0.12**</td>
<td>1.21±0.16**</td>
<td>1.73±0.09**</td>
<td>1.86±0.20**</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.15±0.03**</td>
<td>0.14±0.05**</td>
<td>0.11±0.03**</td>
<td>0.24±0.06**</td>
</tr>
<tr>
<td>12a</td>
<td>0.38±0.09**</td>
<td>0.59±0.07**</td>
<td>0.61±0.07**</td>
<td>1.12±0.18**</td>
</tr>
<tr>
<td>12b</td>
<td>0.46±0.02**</td>
<td>0.44±0.07**</td>
<td>0.76±0.08**</td>
<td>1.06±0.12**</td>
</tr>
<tr>
<td>12c</td>
<td>0.44±0.11**</td>
<td>0.42±0.06</td>
<td>0.67±0.04**</td>
<td>1.08±0.03**</td>
</tr>
<tr>
<td>12d</td>
<td>0.46±0.06**</td>
<td>1.13±0.06**</td>
<td>0.97±0.07**</td>
<td>0.54±0.03**</td>
</tr>
<tr>
<td>12e</td>
<td>0.22±0.07**</td>
<td>0.35±0.04**</td>
<td>0.65±0.07**</td>
<td>1.04±0.06**</td>
</tr>
<tr>
<td>12f</td>
<td>0.22±0.10**</td>
<td>0.31±0.15</td>
<td>0.73±0.17**</td>
<td>1.05±0.3**</td>
</tr>
<tr>
<td>13a</td>
<td>1.09±0.10</td>
<td>1.16±0.09**</td>
<td>0.92±0.09**</td>
<td>0.73±0.13**</td>
</tr>
<tr>
<td>13b</td>
<td>0.34±0.08**</td>
<td>0.44±0.06**</td>
<td>0.49±0.09**</td>
<td>0.79±0.09**</td>
</tr>
<tr>
<td>13c</td>
<td>0.94±0.09</td>
<td>0.40±0.09**</td>
<td>0.51±0.07**</td>
<td>1.03±0.11**</td>
</tr>
<tr>
<td>13d</td>
<td>0.43±0.05**</td>
<td>0.61±0.07**</td>
<td>0.74±0.13**</td>
<td>0.80±0.11**</td>
</tr>
<tr>
<td>13e</td>
<td>0.56±0.06**</td>
<td>0.54±0.04</td>
<td>0.96±0.13**</td>
<td>0.36±0.07**</td>
</tr>
<tr>
<td>13f</td>
<td>0.38±0.04**</td>
<td>0.79±0.03</td>
<td>1.10±0.11**</td>
<td>1.03±0.19**</td>
</tr>
</tbody>
</table>

**Significantly different compared to respective control values, *P* < 0.01.

*Dose level: test compounds (50 mg/kg body wt), indomethacin (10 mg/kg body wt).*

*Values are expressed as mean ± SEM (no. of animals = 6) and analyzed by ANOVA.

*c values in parentheses (percentage anti-inflammatory activity, AI%).

In the present investigation amongst twelve compounds tested, compound 13e was found to exhibit the best AI activity with 81% inhibition but not superior to the reference drug indomethacin (87% inhibition) while compound 12d exhibited good AI activity (71% inhibition) 4h after carrageenan injection. Compounds 13a, 13b and 13d also showed significant AI activity with 58-61% inhibition after 4h. Compounds 13b and 13c showed good AI activity with ≥ 70% inhibition when compared to reference drug indomethacin (94% inhibition) 3h after carrageenan injection whereas compounds 12a-c, 12e-f and 13d showed significant AI activity with 56-65%
inhibition after 3h. A careful analysis of the AI activity results showed that in case of five compounds 12d, 13a, 13d-f the AI activity increases after 4h as compared to after 3h which suggest that these compound were not easily metabolized in the system while seven compounds 12a-c, 12e-f and 13b-c were unable to maintain their AI activity after 4h indicating their easy metabolism in the system.

5.3.3.2. Antimicrobial activity

Most of the tested chemical compounds exhibited moderate antibacterial activity against both the Gram-positive (Staphylococcus aureus and Bacillus subtilis) bacteria (Table 5.3). However, all of the tested compounds were found to be inactive against Gram-negative bacteria (E. coli and P. aeruginosa). On the basis of zone of inhibition, compound 13f was found to be the most effective against S. aureus and B. subtilis with zone of inhibition of 18.6 mm (MIC 64 µg/mL) and 20.6 mm (MIC 32 µg/mL) respectively. Compounds 12d, 13d and 13e also exhibited zone of inhibition 19.6 mm (MIC 64 µg/mL), 18.6 mm (MIC 64 µg/mL), and 19.3 mm (MIC 64 µg/mL) respectively, against B. subtilis. However in case of fungi, compounds 13f and 12c were found to be the best in inhibiting the growth of yeast, S. cerevisiae with zone of inhibition >15.0 mm with MIC value 64 µg/mL when compared to the reference drug amphotericin-B with zone of inhibition 19.3 mm with MIC 12.5 µg/mL (Table 5.2). In the whole series, the MIC of various tested chemical compounds ranged between 64 µg/mL and 256 µg/mL against Gram positive bacteria and between 64 µg/mL and 128 µg/mL against tested yeast.

Amongst all the tested compounds, 13f was found to show good activity against both the tested bacterial and fungal pathogens. From the present investigation it is clear that the compounds having halogens as substituent possesses better antibacterial as well as antifungal activity in comparison to methyl and methoxy substituents.

Table 5.3. In vitro antimicrobial activity of 12 and 13 using agar well diffusion method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of zone of inhibition in mm$^2$ (MIC)$^\text{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>12a</td>
<td>15.3 (128)</td>
</tr>
<tr>
<td>12b</td>
<td>14.6 (256)</td>
</tr>
<tr>
<td>12c</td>
<td>16.6 (128)</td>
</tr>
<tr>
<td></td>
<td>12d</td>
</tr>
<tr>
<td>----</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>17.6 (64)</td>
</tr>
<tr>
<td></td>
<td>15.3 (128)</td>
</tr>
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<td></td>
<td>13.6 (256)</td>
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<td>15.6 (128)</td>
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<td>16.3 (128)</td>
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<td>14.6 (256)</td>
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<td>16.6 (128)</td>
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<tr>
<td></td>
<td>17.3 (64)</td>
</tr>
<tr>
<td></td>
<td>18.6 (64)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxaclin</td>
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<td></td>
<td>Amphotericin-B</td>
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</tbody>
</table>

* Values, including diameter of the well (8mm), are means of three replicates.

b Values in parentheses; MIC (µg/mL) = minimum inhibitory concentration.

Nt = Not tested; 
- No activity.

5.4. Conclusions

In the present investigation twelve compounds (12 and 13) have been synthesized and evaluated for their in vivo AI and in vitro antimicrobial (antibacterial and antifungal) activity. In case of AI assay, compound 13e bearing sulfonamide and chloro moiety was found to exhibit the best AI activity with 81% inhibition in comparison to the reference drug indomethacin with 87% inhibition 4h after carrageenan injection. In case of compounds 12d, 13a, 13d-f AI activity increases after 4h as compared to after 3h suggesting that these compounds were not easily metabolized in the system. From the AI results it seems that compounds bearing electron withdrawing (halogen) substituents were better AI agents in comparison to compounds bearing electron donating (methyl and methoxy) groups. In case of antimicrobial assay compound 13f was found to show good activity against both the tested bacterial (gram positive) and fungal pathogens (S. cerevisiae). It is pertinent to mention here that newly synthesized compounds did not show any antibacterial activity against both the gram negative bacterial strains as well as antifungal activity against Candida albicans used in the study. Compounds having electron withdrawing substituents (halogens) exhibited good antibacterial as well as antifungal activity in comparison to compounds having electron donating substituents (methyl and methoxy). Efforts are underway for the newly synthesized compounds 12 and 13 to be evaluated for their in vivo anti-cancer activity.
5.5. Experimental Section

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on ABB MB 3000 DTGS IR instrument using the KBr pellet technique. $^1$H NMR and $^{13}$C NMR spectra were recorded either in pure DMSO-$d_6$ or in CDCl$_3$ on Bruker NMR spectrometers at 300 MHz and 75.5 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in $\delta$, ppm. $^1$H NMR data are reported in order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; ex, exchangeable by D$_2$O), approximate coupling constant in Hertz, number of protons and type of protons. The purity of the compounds was checked by $^1$H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Mass spectra were recorded on JEOL-AccuTOF JMS-T100LC Mass spectrometer having DART (Direct Analysis in Real Time) source in ES$^+$ mode.

5.5.1. General procedure for synthesis of 2-(1,3-diaryl-1H-pyrazol-4-yl)-1H-benzo[d]imidazoles (12 and 13)

To a solution of 4-formylpyrazole (1 mmol) and ortho-phenylenediamine (1.2 mmol) in 10 ml of dimethylformamide was added oxone (1 mmol) and 1 mL of water. The reaction mixture was refluxed for 1-2 h when colour of reaction changed from greenish to reddish brown. The course of reaction was monitored by thin layer chromatography. Reaction mixture was poured in water whereupon a brown solid precipitated out. The solid so precipitated out was filtered, washed with hot water and dried to afford the target compound in good yield.

2-(1,3-phenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (12a)

m.p. 188-190 \(^\circ\)C, yield 84%; IR (cm$^{-1}$): 1597 (s, C=N stretch), 1504 (m, N-H bend); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 9.11 (s, 1H, C$_5$-H pyrazol), 7.98 (d, $J$ = 7.8 Hz, 2H, Ar-H), 7.89 (d, $J$ = 6.9 Hz, 2H, Ar-H), 7.61-7.59 (m, 4H, Ar-H, Benzim-H), 7.44-7.42 (m, 4H, Ar-H), 7.24 (dd, $J$ = 5.7, 3.3 Hz, 2H, Benzim-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 150.7, 145.6, 142.4, 141.2, 138.5, 132.6, 131.1, 129.7, 128.7, 128.6, 128.1, 122.7, 119.1, 115.3, 113.5; DART MS: m/z 337 ([M+H]$^+$), C$_{22}$H$_{16}$N$_4$H$^+$ Calcd. 337.
2-(1-phenyl-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (12c)

m.p. 142-144 °C, yield 85%; IR (cm\(^{-1}\)): 1597 (s, C=N stretch), 1512 (N-H bend); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 9.05 (s, 1H, C\(_5\)-H pyrazol), 7.96 (d, \(J = 8.1\) Hz, 2H, Ar-H), 7.86 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.61-7.57 (m, 4H, Ar-H, Benzim-H), 7.44-7.33 (m, 2H, Ar-H), 7.22 (dd, \(J = 6.0\), 3.3 Hz, 2H, Benzim-H), 7.00 (d, \(J = 8.7\) Hz, 2H, Ar-H), 3.80 (s, 3H, OCH\(_3\)); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 159.9, 150.8, 149.3, 146.1, 139.3, 130.2, 129.8, 129.3, 127.5, 122.6, 119.2, 114.2, 112.5, 111.9, 55.5 (OCH\(_3\)); DART MS: \(m/z\) 367 ([M+H]\(^+\)), C\(_{23}\)H\(_{18}\)N\(_4\)O\(_3\) Calcd. 367.

2-(1-phenyl-3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (12d)

m.p. 260-262 °C, yield 82%; IR (cm\(^{-1}\)): 3148 (m, N-H stretch), 1597 (s, C=N stretch), 1512 (N-H bend); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 9.30 (s, 1H, C\(_5\)-H pyrazol), 7.96 (d, \(J = 7.5\) Hz, 2H, Ar-H), 7.91-7.86 (m, 2H, Ar-H), 7.70 (dd, 6.0, 3.0 Hz, 2H, Benzim-H), 7.61 (t, \(J = 7.5\) Hz, 2H, Ar-H), 7.47-7.44 (m, 1H, Ar-H), 7.22 (dd, \(J = 6.0\), 3.0 Hz, 2H, Benzim-H), 7.30 (t, \(J = 8.7\) Hz, 2H, Ar-H); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 162.9 (d, \(^1\)J\(_{CF}\) = 246.9 Hz), 150.4, 146.1, 141.9, 139.1, 135.1, 132.2, 130.8 (d, \(^1\)J\(_{CF}\) = 8.3 Hz), 130.3, 128.0, 124.6, 119.4.
116.1 (d, \( ^2J_{CF} = 21.1 \) Hz), 114.8, 112.2; DART MS: \( m/z \) 355 ([M+H\(^+\)], C\(_{22}H_{15}N_4F\)H\(^+\)
Calcd. 355.

**2-(1-phenyl-3-(4-chlorophenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (12e)**

m.p. 168-170 °C, yield 86%; IR (cm\(^{-1}\)): 3101 (m, N-H stretch), 1597 (C=N stretch), 1504 (N-H bend); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta \) 9.12 (s, 1H, C\(_5\)-H pyrazole), 7.97 (d, \( J = 8.1 \) Hz, 4H, Ar-H), 7.61-7.57 (m, 4H, Ar-H, SO\(_2\)N\(_2\)H\(_2\)), 7.51 (d, \( J = 8.4 \) Hz, 2H, Ar-H), 7.22 (dd, \( J = 6.0, 3.3 \) Hz, 2H, Benzim-H); \(^13\)C NMR (75.5 MHz, DMSO-\(d_6\)): \( \delta \) 150.5, 145.3, 142.6, 141.3, 133.9, 131.5, 131.1, 130.5, 128.8, 128.0, 122.8, 119.1, 115.4, 113.6; DART MS: \( m/z \) 371/373 ([M+H/M+2+H\(^+\)], C\(_{22}H_{15}N_4Cl\)H\(^+\)
Calcd. 371/373.

**2-(1-phenyl-3-(4-bromophenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (12f)**

m.p. 184-188 °C, yield 80%; IR (cm\(^{-1}\)): 3140 (N-H, stretch), 1597 (C=N stretch), 1512 (N-H bend); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta \) 9.13 (s, 1H, C\(_5\)-H pyrazole), 7.98 (m, 4H, Ar-H), 7.61-7.57 (m, 4H, Ar-H), 7.51 (d, \( J = 8.4 \) Hz, 2H, Ar-H), 7.46-7.39 (m, 1H, Ar-H), 7.25 (dd, \( J = 6.0, 3.3 \) Hz, 2H, Benzim-H); \(^13\)C NMR (75.5 MHz, DMSO-\(d_6\)): \( \delta \) 149.8, 145.6, 139.3, 133.7, 131.4, 131.0, 130.4, 130.2, 128.7, 127.7, 122.8, 119.2, 118.9, 115.2, 112.5; DART MS: \( m/z \) 415/417 ([M+H/M+2+H\(^+\)], C\(_{22}H_{15}N_4Br\)H\(^+\)
Calcd. 415/417.

**2-[1-(4-aminosulfonylphenyl)-3-phenyl-1H-pyrazol-4-yl]-1H-benzo[d]imidazole (13a)**

m.p. 130-132 °C, yield 86%; IR (cm\(^{-1}\)): 1597 (s, C=N stretch), 1504 (N-H bend), 1335 & 1157 (s, SO\(_2\) stretch); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta \) 9.21 (s, 1H, C\(_5\)-H pyrazole), 8.19 (d, \( J = 8.4 \) Hz, 2H, Ar-H), 8.04 (d, \( J = 8.7 \) Hz, 2H, Ar-H), 7.90-7.88 (m, 2H, Ar-H), 7.60 (dd, \( J = 6.0, 3.3 \) Hz, 2H, Benzim-H), 7.49 (s, ex, 2H, SO\(_2\)N\(_2\)H\(_2\)), 7.45-7.43 (m, 3H, Ar-H), 7.24 (dd, \( J = 6.0, 3.3 \) Hz, 2H, Benzim-H); \(^13\)C NMR (75.5 MHz, DMSO-\(d_6\)): \( \delta \) 151.7,
2-{1-(4-aminosulfonylphenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl}-1H-benzo[d]imidazole (13b)

m.p. 192-194 °C, yield 87%; IR (cm⁻¹): 1597 (s, C=O stretch), 1512 (N-H bend), 1335 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 9.20 (s, 1H, C₅-H pyrazol), 8.17 (d, J = 8.7 Hz, 2H, Ar-H), 8.02 (d, J = 8.7 Hz, 2H, Ar-H), 7.76 (d, J = 8.1 Hz, 2H, Ar-H), 7.60 (dd, J = 6.0, 3.3 Hz, 2H, Benzim-H) 7.49 (s, ex, 2H, SO₂NH₂), 7.27-7.23 (m, 4H, Ar-H, Benzim-H), 2.35 (s, 3H, CH₃); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 151.7, 145.6, 142.5, 141.5, 138.6, 131.4, 129.4, 128.5, 127.9, 127.2, 122.7, 119.0, 115.2, 113.3, 21.3 (CH₃); DART MS: m/z 430 ([M+H]⁺), C₂₃H₁₉N₅O₂S⁺ Calcd. 430.

2-{1-(4-aminosulfonylphenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl}-1H-benzo[d]imidazole (13c)

m.p. 172-174 °C, yield 83%; IR (cm⁻¹): 1597 (s, C=O stretch), 1512 (s, N-H bend), 1366 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 9.18 (s, 1H, C₅-H pyrazol), 8.16 (d, J = 8.7 Hz, 2H, Ar-H), 8.02 (d, J = 8.7 Hz, 2H, Ar-H), 7.85 (d, J = 8.4 Hz, 2H, Ar-H), 7.60 (dd, J = 6.0, 3.3 Hz, 2H, Benzim-H), 7.48 (s, ex, 2H, SO₂NH₂), 7.24 (dd, J = 6.0, 3.0 Hz, 2H, Benzim-H), 7.01 (d, J = 8.7 Hz, 2H, Ar-H), 3.79 (s, 3H, OCH₃); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 160.2, 151.4, 145.7, 142.4, 141.5, 131.4, 130.0, 127.9, 124.6, 122.7, 118.9, 115.2, 114.2, 113.1, 55.6 (OCH₃); DART MS: m/z 446 ([M+H]⁺), C₂₃H₁₉N₅O₃S⁺ Calcd. 446.

2-{1-(4-aminosulfonylphenyl)-3-(4-flourophenyl)-1H-pyrazol-4-yl}-1H-benzo[d]imidazole (13d)

m.p. 202-204 °C, yield 90%; IR (cm⁻¹): 1597 (s, C=N stretch), 1512 (N-H bend), 1366 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 9.22 (s, 1H, C₅-H pyrazol), 8.17 (d, J = 8.1 Hz, 2H, Ar-H),
8.04 (m, 4H, Ar-H), 7.59 (m 2H, Ar-H), 7.49 (s, ex, 2H, SO₂NH₂), 7.32-7.24 (m, 4H, Ar-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 162.9 (d, ¹JC₅ = 246.9 Hz), 150.7, 145.5, 142.6, 141.4, 131.4, 130.9 (d, ¹JC₅ = 8.3 Hz), 128.8, 128.0, 122.6, 119.0, 115.6 (d, ²JC₅ = 21.9 Hz), 113.6; DART MS: m/z 434 ([M+H]⁺), C₂₂H₁₈N₃O₂SFH⁺ Calcd. 434.

2-[(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-1H-pyrazol-4-yl]-1H-benzo[d]imidazole (13e)
m.p. 188-190 °C, yield 84%; IR (cm⁻¹): 1597 (C=N stretch), 1512 (N-H bend), 1335 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 9.22 (s, 1H, C₅-H pyrazol), 8.17 (d, J = 9.0 Hz, 2H, Ar-H), 8.03 (d, J = 8.7 Hz, 2H, Ar-H), 7.95 (d, J = 8.4 Hz, 2H, Ar-H), 7.60 (dd, J = 6.0, 3.3 Hz, 2H, Ar-H), 7.53 (d, J = 8.4 Hz, 2H, Ar-H), 7.50 (s, ex, 2H, SO₂NH₂), 7.24 (dd, J = 6.0, 3.0 Hz, 2H, Ar-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 150.4, 145.4, 142.7, 141.4, 131.4, 130.9, 128.8, 128.0, 122.7, 119.1, 115.2, 113.7; DART MS: m/z 450/452 ([M+H/M+2+H]⁺), C₂₂H₁₈N₃O₂SCIH⁺ Calcd. 450/452.

2-[(4-aminosulfonylphenyl)-3-(4-bromophenyl)-1H-pyrazol-4-yl]-1H-benzo[d]imidazole (13f)
m.p. 178-180 °C, yield 79%; IR (cm⁻¹): 1597 (C=N stretch), 1504 (N-H bend), 1335 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 9.22 (s, 1H, C₅-H pyrazol), 8.17 (d, J = 8.4 Hz, 2H, Ar-H), 8.03 (d, J = 8.7 Hz, 2H, Ar-H), 7.89 (d, J = 8.4 Hz, 2H, Ar-H), 7.66 (d, J = 8.4 Hz, 2H, Ar-H), 7.59 (dd, J = 5.7, 3.0 Hz, 2H, Ar-H), 7.50 (s, ex, 2H, SO₂NH₂), 7.23 (dd, J = 5.7, 3.0 Hz, 2H, Ar-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 150.5, 145.4, 142.7, 141.4, 131.4, 130.8, 128.1, 128.0, 122.7, 119.1, 115.4, 113.2; DART MS: m/z 494/496 ([M+H/M+2+H]⁺), C₂₂H₁₆N₃O₂SBrH⁺ Calcd. 494/496.
5.6. References


25 Ahmad, I.; Beg, A. J. J. Ethnopharmacol. 2001, 74, 113-123.
