Antifungal activity and theoretical study of synthesized pyrazole-imidazole hybrids

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Abstract. The density functional theory (DFT) and thermodynamic analyses were applied to study the stability and chemical reactivity of the synthesized CMPIP {2-chloro-4-(4-(1-methyl-1H-pyrazole-4-yl)-1H-imidazole-5-yl)pyridine} and MPIMPPA {4-(4-(1-methyl-1H-pyrazole-4-yl)-1H-imidazole-5-yl)-N-(4-morpholino phenyl)pyridin-2-amine}. The results concluded that the CMPIP compound is more stable than MPIMPPA. The antifungal study was confirmed that the MPIMPPA has a higher inhibition zone against Aspergillus niger (A. niger), as compared to the standard drug used, while the CMPIP compound showed weaker activity than the positive control.

1. Introduction.
Heterocyclic chemistry is of great interest due to its application in the development of medicinal chemistry and drug discovery [1]. Azole heterocycles have a broad spectrum of therapeutical and pharmacological activities and represent building blocks in the structures of various natural products [2]. Pyrazole molecules contain five-membered rings, which belong to the azole family. Various strategies used to synthesize pyrazole derivatives have been described in the literature in order. Therapeutic applications of pyrazole derivatives have been tested against cancer, contagious (i.e., AIDS), non-communicable (i.e., malaria), and neurodegenerative (i.e., Parkinson's and Alzheimer) diseases [3]. A wide range of potential bioactivities of pyrazoles has been reported, involving anticancer [4], anti-inflammatory [5], antimicrobial [6], antioxidant [7], and antileishmanial [8] ones. Furthermore, pyrazole moieties have been found to be versatile building blocks in drug discovery and medicinal chemistry. For instance, Celecoxib (figure 1) is used to treat inflammation, and Rimonabant is used to combat obesity [9].
Figure 1. Chemical structure of Celecoxib.

Pyrazole rings play an important role as sub-units of many natural products, such as Fluviols (A-E) isolated from Pseudomonas fluorescences [10], Formycin obtained from Nocardia interforma, Streptomyces kaniharaensis SF-5573, and Streptomyces sp. MA406-A-1 [11]. On the other hand, a five-membered plane ring, called imidazole, displays anticancer [12], antifungal [13], antibacterial [14], anti-inflammatory [15], and antitubercular [16] bio-activities. Imidazole derivatives are crucial in drug discovery, and many drugs have been developed based on imidazole moiety [17]. For example, clotrimazole's antifungal activity (figure 2) has been used by Bayer AG to treat skin infections such as athlete's foot, jock itch, and ringworm. Alternatively, miconazole and econazole synthesized at Janssen Pharmaceutica exhibited the bio-activity similar to that of clotrimazole in treating skin infections [18].

Figure 2. Chemical structure of clotrimazole and miconazole.

In this study, we have investigated the chemical reactivity and stability of the synthesized pyrazole-imidazole hybrids, and examined their antifungal activity as well.

2. Experimental.
The compounds, including CMPIP and MPIMPPA (figure 3), were synthesized according to the procedure described elsewhere [19].
Figure 3. Synthesis of CMPIP and MPIMPPA pyrazole-imidazole hybrids.

**Reaction reagents and conditions:**
(i) Methyl iodide solution (1.5 equiv.), K₂CO₃ (1.1 equiv.), CH₃CN solution,
(ii) ethyl-1-methyl-1H-pyrazole-4-carboxylate (1.0 equiv.), NaHMDS (2.2 equiv.), THF, 0°C to room temperature, 3 h.,
(iii) SeO₂ (1.1 equiv.), Acetic acid, 70°C, 1.5-2 h.,
(iv) NH₄OAc (10 equiv.), Acetic acid, MW (180°C, 5 min., 15 bar, 250 watt),
(v) p-aminomorpholine (1.5 equiv.), HCl/EtOH (1.0 equiv.), n-Butanol, 160 °C, 16-18 h.

3. Antifungal Activity Studies
Well Diffusion Assay [20] has been used for the antifungal activity test of the compounds, and *A. niger* cultured on potato dextrose agar was utilized to measure *in vitro* growth inhibitory activity of the synthesized compounds. DMSO was used as a solvent for the preparation of the stock solution (10⁻² mol. L⁻¹) of the tested compounds in a concentration of 200 ppm. Positive control involving Amphotericin B was used for comparison. A period of 72 h at 30 °C was incubated for the compounds' solutions in DMSO, which were placed onto the cultured agar medium. The experiments were repeated three times for observation of inhibition zones.

4. Results and Discussion

4.1. 3D Molecular Modeling Studies
The molecular modeling investigation was used to gather more information on the synthesized compounds. Becke's three parameters and Lee-Yang-Parr functional (B3LYP) [21] level with 6-311G (d) basis set (figure 4) were optimized for the CMPIP and MIIMPPA compounds.
Figure 4. Optimized geometry of CMPIP and MPIMPPA.

The calculations and observations were performed using the Gaussian 09W program platform and analyzed with Gauss View 5.0.9 program. Various bond lengths and angles have been observed for the whole compounds, as shown in Table 1.

| Bond length (Å) | Bond angle (°) | Bond length (Å) | Bond angle (°) |
|-----------------|----------------|-----------------|----------------|
| N₁-N₂ (1.380)   | N₁-N₂-C₃ (104.63) | N₁-N₂ (1.427)  | N₁-N₂-C₃ (109.73) |
| N₂-C₃ (1.341)   | N₂-C₃-C₄ (111.88) | N₂-C₃ (1.502)  | N₂-C₃-C₄ (108.83) |
| C₃-C₄ (1.423)   | C₃-C₄-C₅ (111.79) | C₃-C₄ (1.516)  | C₃-C₄-C₅ (105.71) |
| C₄-C₅ (1.366)   | C₄-C₅-C₆ (128.64) | C₄-C₅ (1.332)  | C₄-C₅-C₆ (112.54) |
| C₅-C₆ (1.454)   | C₅-C₆-C₇ (110.55) | C₅-C₆ (1.471)  | C₅-C₆-C₇ (106.36) |
| N₁₀-N₁₁ (1.401) | N₁₁-C₁₂-N₁₃ (111.09) | N₁₁-N₁₂ (1.303) | N₁₁-N₁₂-N₁₃ (111.63) |
| N₁₁-N₁₂ (1.325) | N₁₂-C₁₃-C₁₄ (132.09) | C₁₂-C₁₃ (1.475) | C₁₂-C₁₃-C₁₄ (119.29) |
| C₁₃-C₁₄ (1.369) | C₁₄-C₁₅-C₁₆ (118.95) | C₁₃-C₁₄ (1.347) | C₁₃-C₁₄-C₁₅ (120.92) |
| C₁₆-C₁₇ (1.407) | C₁₇-C₁₈-C₁₉ (120.60) | C₁₆-C₁₇ (1.349) | C₁₆-C₁₇-C₁₈ (119.38) |
| C₁₇-C₁₈ (1.390) | C₁₈-C₁₉-C₂₀ (119.47) | C₁₇-C₁₈ (1.539) | C₁₇-C₁₈-C₂₀ (120.43) |
| C₁₈-C₁₉ (1.412) | C₁₉-C₂₀-N₂₁ (125.27) | C₁₈-C₁₉ (1.534) | C₁₈-C₁₉-N₂₁ (120.03) |
| C₁₉-C₂₀ (1.387) | C₂₀-N₂₁ (1.298) | C₁₉-C₂₀ (1.355) | C₂₀-N₂₁-N₂₂ (108.58) |
| C₂₀-N₂₁ (1.318) | C₂₀-N₂₁ (1.469) | C₂₀-N₂₁ (1.298) | C₂₀-N₂₁-N₂₂ (120.46) |
| C₂₁-N₂₂ (1.469) | C₂₁-N₂₂ (1.469) | C₂₁-N₂₂ (1.469) | C₂₁-N₂₂ (1.469) |
| C₂₂-C₂₃ (1.450) | C₂₂-C₂₃ (1.450) | C₂₂-C₂₃ (1.450) | C₂₂-C₂₃ (1.450) |
| C₂₃-C₂₄ (1.540) | C₂₃-C₂₄ (1.540) | C₂₃-C₂₄ (1.540) | C₂₃-C₂₄ (1.540) |
| C₂₄-C₂₅ (1.349) | C₂₄-C₂₅ (1.349) | C₂₄-C₂₅ (1.349) | C₂₄-C₂₅ (1.349) |
| C₂₅-C₂₆ (1.535) | C₂₅-C₂₆ (1.535) | C₂₅-C₂₆ (1.535) | C₂₅-C₂₆ (1.535) |
| C₂₆-C₂₇ (1.356) | C₂₆-C₂₇ (1.356) | C₂₆-C₂₇ (1.356) | C₂₆-C₂₇ (1.356) |
| C₂₇-C₂₈ (1.500) | C₂₇-C₂₈ (1.500) | C₂₇-C₂₈ (1.500) | C₂₇-C₂₈ (1.500) |
| C₂₈-C₂₉ (1.534) | C₂₈-C₂₉ (1.534) | C₂₈-C₂₉ (1.534) | C₂₈-C₂₉ (1.534) |
| C₂₉-C₃₀ (1.375) | C₂₉-C₃₀ (1.375) | C₂₉-C₃₀ (1.375) | C₂₉-C₃₀ (1.375) |
| C₃₀-C₃₁ (1.389) | C₃₀-C₃₁ (1.389) | C₃₀-C₃₁ (1.389) | C₃₀-C₃₁ (1.389) |
The importance of the theoretical approach is to explain the chemical reactivity and selection of the chemically active sites of the compounds during the reaction. According to Frontier Molecular Orbitals approach, the energy gap between HOMO and LUMO orbitals explains the electron transfer interaction [22]. Some parameters, such as global hardness (η), potential ionization (I), chemical potential (μ), global electrophilicity index (ω), electronegativity (χ), and global softness (S), are called chemical reactivity values [23].

\[ \chi = -\frac{E_{LUMO} + E_{HOMO}}{2} \]
\[ \mu = -\chi = \frac{E_{LUMO} + E_{HOMO}}{2} \]
\[ \eta = \frac{E_{LUMO} - E_{HOMO}}{2} \]
\[ S = \frac{1}{2\eta} \]
\[ \omega = \frac{\mu^2}{2\eta} \]
\[ \sigma = \frac{1}{\eta} \]

To clarify the compound chemical reactivity, the orbital energy comparative analysis is required. The HOMO orbital energy always acts as an "electron donor" orbital, which possesses higher energy. The LUMO orbital always acts as an "electron acceptor" orbital, which possesses lower energy (table 2).

| Parameters | MPIMPPA | CMPIP |
|------------|---------|-------|
| \( E_{HOMO} (eV) \) | -0.18599 | -0.22295 |
| \( E_{LUMO} (eV) \) | -0.05268 | -0.07371 |
| \( \Delta E_{gap} (eV) \) | 0.13331 | 0.14924 |
| \( I_E (eV) \) | 0.18599 | 0.22295 |
| \( A (eV) \) | 0.05268 | 0.07371 |
| \( \eta (eV) \) | 0.066655 | 0.07462 |
| \( \omega (eV) \) | 0.1068246 | 0.1474249 |
| \( \chi (eV) \) | -0.119335 | -0.148330 |
| \( \mu (eV) \) | 0.119335 | 0.148330 |
| \( S (eV) \) | 7.50131 | 6.70061 |
| \( \sigma (eV) \) | 15.00263 | 13.40123 |

Based on the results listed in Table 2, MPIMPPA with a higher HOMO energy (\( E_{HOMO} = -0.18599 \) eV), lower potential ionization value (\( I = 0.18599 \) eV), and lower electron affinity value (\( A = 0.05268 \) eV) acts as a better electron donor, while CMPIP with a lower LUMO energy (\( E_{LUMO} = -0.07371 \) eV), higher electron affinity (\( A = 0.07371 \) eV), and higher potential ionization value (\( I = 0.22295 \) eV) acts as a better electron acceptor. Chemical hardness (softness) indicates that MPIMPPA (\( \eta = 0.07462 \) eV, \( S = 6.70061 \) eV) is more reactive than CMPIP (\( \eta = 0.066655 \) eV, \( S = 7.50131 \) eV). CMPIP (\( \omega = 0.1474249 \) eV) has stronger
electrophilic properties than MPIMPPA ($\omega = 0.1068246 \text{ eV}$), according to the results on the electrophilicity index ($\omega$). A smaller frontier orbital gap ($\Delta E = 0.13331 \text{ eV}$) of MPIMPPA (figure 5) results in a higher chemical reactivity, more polarizable and less kinetically stable "soft molecule" form.

![Figure 5. HOMO and LUMO molecular orbital energy gap of MPIMPPA.](image)

**5. Thermodynamic Study**
The energy required for the formation of derivatives was investigated. The respective results are listed in table 3.

| Compound | $\Delta H_f$ Kcal/mol | $\Delta S$ Kcal/mol.k | $\Delta G$ Kcal/mol |
|----------|------------------------|------------------------|---------------------|
| MPIMPPA  | 287.82                 | 179.00                 | -1310.18            |
| CMPIP    | 141.40                 | 127.53                 | -1197.01            |

The data on the heat of formation indicate the compound CMPIP can be formed at lower energy ($\Delta H_f = 141.40 \text{ Kcal/mol}$) than MPIMPPA ($\Delta H_f = 287.82 \text{ Kcal/mol}$). The difference between the heat of formation and the stability level confirms that the product formation reaction proceeded and was controlled thermodynamically. The higher degree of freedom (higher value of $\Delta S$) of CMPIP, as compared to that of MPIMPPA, proves that the formation of CMPIP can be easier and faster than that of MPIMPPA, according to results in table 3.

**6. Antifungal activity**
Antifungal activities against fungal strains named *A. niger* of the two compounds were tested, and both compounds displayed a considerable activity, compared to the positive control of Amphotericin B, as shown in table 4.
Table 4. Antifungal activity data for CMPIP and MPIMPPA.

| Compound     | Zone of Inhibition (cm) [Conc (200 ppm)] ± SD |
|--------------|-----------------------------------------------|
| CMPIP        | 1.30 ± 0.03                                   |
| MPIMPPA      | 1.70 ± 0.06                                   |
| Amphotericin B | 1.50 ± 0.02                                   |

The CMPIP compound showed a weak activity, as compared to Amphotericin B, with an inhibition zone of 1.30 ± 0.03 cm against *A. niger*, while the MPIMPPA showed a potent activity with an inhibition zone of 1.70 ± 0.06 cm. The increase in activity of the MPIMPPA might be due to the hydrogen-bonding formation through the nitrogen atom (NH) with the "active centers" of cell constituents leading to interference with the normal cell process [24] or due to the exciting of morpholine moiety, which plays an important role in increasing the activity of compounds [25].

7. Conclusion

Theoretical studies, including DFT and thermodynamic analyses, confirmed the chemical reactivity and stability of both synthesized compounds. The obtained results provided the thermodynamical substantiation of the fact that the CMPIP intermediate compound can be formed easier and faster than the PIMPPA product compound. The results also proved higher chemical reactivity and lower kinetic stability of the MPIMPPA target compound, according to its frontier orbital gap. The antifungal study revealed an excellent activity of MPIMPPA and a weak activity of CMPIP, as compared to the standard control of Amphotericin B.

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