Study on regioselective synthesis of bioactive bis-spiropyrazolines using molecular orbital calculations

Thoraya Abd El-Reheem Farghaly *, Ikhlass Mohamed Abbas, Walid Mohamed Ibrahim Hassan and Mai Samir Lotfy

Department of Chemistry, Faculty of Science, Cairo University, Giza, 12613, Egypt

*Corresponding author at: Department of Chemistry, Faculty of Science, Cairo University, Giza, 12613, Egypt.
Tel.: +2.02.35676608, Fax: +2.02.35676501, E-mail address: thoraya@hotmail.com (T.A.E. Farghaly).

ABSTRACT

1,3-Dipolar cycloaddition reaction of \(2E,2′E\)-2,2′-(1,4-phenylene bis(methanylylidene)) bis(3,4-dihydronaphthalen-1(2H)-one) \(3\) and \(2E,2′E\)-2,2′-(1,4-phenylene bis(methanylylidene)) bis(1H-indene-1,3(2H)-dione) \(8\) with a variety of nitrilimines, generated in situ by triethylamine dehydrohalogenation of the corresponding hydrazonyl halides, \(4\) proceeded region-selectively and affording novel spiropyrazoline derivatives \(5\) and \(10\), respectively. The mechanisms of the reactions studied are discussed and the structures of the products were confirmed by spectral data and elemental analyses. Also, molecular orbital plots for HOMO and LUMO verify our suggested mechanism and stereo-selectivity of the reaction. The antimicrobial activity of the products was evaluated and promising results were obtained.

1. Introduction

Spiro and bis-spiro-heterocyclic compounds represent an important class of substances characterized by highly pronounced biological properties \([1-4]\). The most developed phenomenon for the synthesis of these compounds depends mainly on cycloaddition reaction to exocyclic double bonds \([5-9]\). 1,3-Dipolar cycloaddition reactions are considered the most successful process for the construction of five-membered ring containing spiro and bis-spiro-compounds due to high regio- and stereo-selective properties of these reactions \([10,11]\). From this reaction, pyrazolines derivatives are one of the synthesized compounds. These compounds have been found to exhibit considerable biological activities such as antibacterial \([12-16]\), antifungal \([15-17]\), antiviral \([15]\), anti-inflammatory \([18-23]\), analgesic \([19]\), and antidepressant ones \([24]\).

In the present work and in continuation to our previous work concerning with reactions of nitrilimines \([25-29]\), we investigate the synthesis of spiropyrazoline-containing compounds through 1,3-dipolar cycloaddition reaction of nitrilimines to various bis-exocyclic olefinic linkage containing compounds. This investigation will allow not to study the regiochemistry of 1,3-dipolar cycloaddition reaction at neighboring olefinic linkages, but also to prepare novel bis-spiro pyrazoline containing compounds with an element of symmetry which is a characteristic property of many biologically active natural and synthetic compounds \([30]\). Also, we interested to study the mechanism of this reaction since in the absence of quantum chemical calculations for this mechanism, the product can be verified by spectroscopic methods, but give no reason for the stereo-selectivity of the reaction. In this study we have performed quantum chemical calculations to investigate why one of these mechanisms are favored over the other.

2. Experimental

2.1. Chemistry

2.1.1. Instrumentation

Melting points were measured on Electrothermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3500 and Shimadzu FTIR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (\(^1H\) NMR) or 100 MHz (\(^{13}C\) NMR) and run in deuterated dimethylsulphoxide (DMSO-d$_6$).
Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectro-meter at 70 eV. Elemental analyses were measured by using a German made Elementar Vario LIII CHNS analyzer. The starting compounds 3, 8 and hydrazonoyl chlorides were prepared as previously described in the literature [31-34].

2.1.2. Reaction of compound 3 and 4

A mixture of compound 3 or 8 (2.5 mmol) and the appropriate hydrazonoyl halides 4a-e (5 mmol) in dry benzene (30 mL) containing triethylamine (7.5 mmol) was heated under reflux for the appropriate time. The reaction mixture was then separated by filtration to remove the triethylamine hydrochloride, then concentrated to 10 mL and cooled overnight. The separated solid was collected and crystallized from ethanol/dioxane (20:80, v/v) and affording the corresponding products 5a-f, 9a-c,f,e or 10a,d (Scheme 1 and 2).

4'-[4-(5'-Phenyl)-1-oxo-5'-(E)-styryl]-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2,3'-pyrazol]-4'-yl(phenyl)-5'-[phenyl]-2'-phenyl-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2,3'-pyrazol]-1-one (5a): Color: Yellow. Yield: 70%. M.p. >300 ºC (KBr, c m⁻¹): 1676 (CO). ¹H NMR (300 MHz, DMSO-d₆, 6 ppm): 1.94 (t, J = 7 Hz; 4H, 2CH₂), 2.87 (t, J = 7 Hz; 4H, 2CH₂), 5.17 (s, 2H, 2CH), 6.83-8.02 (m, 28H, Ar-H), 8.31 (s, 4H, Ar-H).

5c

Scheme 1

5'-[Phenyl]-2'-[4-nitro phenyl]-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2',3'-pyrazol]-4'-yl(phenyl)-5'-[phenyl]-2'-phenyl-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2,3'-pyrazol]-1-one (5d): Color: Brown solid. Yield: 68 %. M.p: 130 ºC. FT-IR (KBr, c m⁻¹): 1677 (CO). ¹H NMR (300 MHz, DMSO-d₆, 6 ppm): 2.50-3.29 (m, 8H, 4CH₂), 5.52 (s, 2H, 2CH), 7.28-8.14 (m, 30H, Ar-H). MS (EI, m/z (%)): 868 (M⁺, 14), 867 (27), 433 (32), 205 (32), 162 (32), 115 (41), 110 (41), 107 (41), 105 (46), 102 (46), 92 (32), 89 (36), 83 (37), 78 (37), 77 (100), 73 (41), 71 (50), 69 (59), 67 (46), 63 (72), 60 (50), 57 (68), 55 (55). Anal. calcld. for: C₃₇H₁₆N₄O₂: C, 74.64; H, 4.64; N, 9.67. Found: C, 74.48; H, 4.50; N, 9.43%.

2'-[4-Nitrophenyl]-4'-[4-(1-oxo-5'-[thiophen-2-yl]-2'-[4-nitro phenyl]-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2',3'-pyrazol]-4'-yl(phenyl)-5'-[phenyl]-2'-phenyl-2',3,4,4'-tetrahydro-1H-spiro[naphthalene-2',3'-pyrazol]-1-one (5e): Color: Dark orange solid. Yield: 69 %. M.p: 220-222 ºC. FT-IR (KBr, c m⁻¹): 1677 (CO). ¹H NMR (300 MHz, DMSO-d₆, 6 ppm): 2.20-3.05 (m, 8H, 4CH₂), 5.41 (s, 2H, 2CH), 6.99-8.14 (m, 26H, Ar-H). MS (EI, m/z (%)): 881 (M⁺+1, 6), 880 (M⁺, 30), 848 (10), 847 (13), 115 (32), 119 (10), 92 (32), 91 (45), 84 (19), 82 (32), 72 (48), 57 (100). Anal. calcld. for: C₃₇H₁₆N₄O₂S: C, 68.17; H, 4.12; N, 9.54. Found: C, 68.02; H, 4.0; N, 9.35%.
5′-(Furan-2-yl)-2′-(4-nitrophenyl)-4′-(4′-(2′-(4-nitrophenyl)-1-oxo-5′-furan-2-yl)-3′,4′-tetrahydroido-1H-spiro[naphthalene-2,3′-pyrazol]-4′-yl)phenyl)-2′,3′,4′,5′-tetrahydrospiro[1H-naphthalene-2,3′-pyrazole]-1,3′-dione (9f): Color: Orange solid. Yield: 81 %. M.p: 212 °C. FT-IR (KBr, v, cm⁻¹): 1746, 1707 (CO). 1H NMR (300 MHz, DMSO-d₆, δ, ppm): 5.34 (s, 1H, CH), 6.76-7.58 (m, 22H, Ar-H), 9.90 (s, 1H, =CH). 13C NMR (100 MHz, CDCl₃, δ, ppm): 61.49, 79.70, 113.77, 123.90, 124.24, 125.26, 125.04, 129.27, 129.91, 130.24, 130.48, 130.69, 131.25, 131.56, 131.69, 137.50, 138.33, 139.21, 140.72, 143.02, 144.71, 193.21, 194.14, 196.34. MS (EI, m/z (%)): 811 (M+1, 1), 800 (M+, 1), 788 (M−1, 1), 776 (M−2, 1), 755 (M−3, 1), 743 (M−4, 1), 721 (M−5, 1), 709 (M−6, 1), 688 (M−7, 1), 667 (M−8, 1), 646 (M−9, 1). Anal. calcd. for: C₁₂₃H₈₂N₁₈O₁₈S: C, 69.74; H, 3.15%; N, 6.49%.

Scheme 2

4′-(2′-(4-Chlorophenyl)-4′-(1,3-dioxo-1H-inden-2(3H)-ylidene)methyl)phenyl)-2′,5′-diaryl-2′-4′-dihydrorydrosiprin[1H-naphthalene-2,3′-pyrazole]-1,3′-dione (9b): Color: Red solid. Yield: 72 %. M.p: >300 °C. FT-IR (KBr, v, cm⁻¹): 1747, 1712 (CO). 1H NMR (300 MHz, DMSO-d₆, δ, ppm): 5.12 (s, 1H, CH), 5.33-6.25 (m, 17H, Ar-H), 6.63-7.16 (m, 17H, Ar-H), 6.23-6.75 (m, 17H, Ar-H), 9.90 (s, 1H, =CH). Anal. calcd. for: C₁₀₂H₇₂N₁₆O₁₄Cl: C, 71.73; H, 3.42%; N, 6.60%.

5′-(5′-(4-Nitrophenyl)-1,3-dioxo-1H-inden-1-ylidene)methyl)phenyl)-5′-phenyl-2′-styryl-2′,4′-dihydrorydrosiprin[1H-naphthalene-2,3′-pyrazole]-1,3′-dione (9c): Color: Orange solid. Yield: 62 %. M.p: >300 °C. FT-IR (KBr, v, cm⁻¹): 1747, 1708 (CO). 1H NMR (300 MHz, DMSO-d₆, δ, ppm): 5.28 (s, 1H, CH), 6.70 (d, J = 12 Hz, 1H, =CH), 6.82-8.19 (m, 24H, Ar-H and =CH). MS (EI, m/z (%)): 610 (M', 2), 481 (100), 104 (58), 77 (94), 75 (45), 60 (50), 57 (45), 51 (32). Anal. calcd. for: C₁₃₂H₇₂N₁₈O₁₈: C, 80.64; H, 4.29; N, 4.59. Found: C, 80.41; H, 4.13; N, 4.26%.

4′-(2′-((3,4-Bis(thiophen-2-yl)-2′,4′-dihydroido[1H-naphthalene-2,3′-pyrazole]-1,3′-dione (10b): Color: Dark green solid. Yield: 60 %. M.p: >300 °C. FT-IR (KBr, v, cm⁻¹): 1748, 1700 (CO). 1H NMR (300 MHz, DMSO-d₆, δ, ppm): 6.65 (d, J = 8 Hz, 4H, Ar-H), 6.88 (d, J = 8 Hz, 4H, Ar-H), 7.64 (s, 4H, Ar-H), 7.42-7.46 and 7.82-8.24 (m, 14H, Ar-H). MS (EI, m/z (%)): 847 (M'+1, 5), 804 (5), 486 (13), 128 (10), 97 (27), 95 (18), 77 (10), 76 (19). Anal. calcd. for: C₄₀H₂₆N₄O₆S: C, 70.58; H, 4.45; N, 9.81%.
Table 1. Shows the total energy, energy of HOMO, energy of LUMO in eV, and energy gap ΔE in eV.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total energy</th>
<th>$E_{\text{HOMO}}$</th>
<th>$E_{\text{LUMO}}$</th>
<th>ΔE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-1300.410665</td>
<td>-0.24639</td>
<td>-0.12643</td>
<td>3.262683</td>
</tr>
<tr>
<td>4a</td>
<td>-1071.861792</td>
<td>-0.20609</td>
<td>-0.05842</td>
<td>4.018352</td>
</tr>
<tr>
<td>4b</td>
<td>-1351.483465</td>
<td>-0.21065</td>
<td>-0.06667</td>
<td>3.917891</td>
</tr>
<tr>
<td>4c</td>
<td>-1149.288269</td>
<td>-0.20122</td>
<td>-0.07758</td>
<td>3.542691</td>
</tr>
<tr>
<td>4d</td>
<td>-1276.420743</td>
<td>-0.22878</td>
<td>-0.09189</td>
<td>3.728941</td>
</tr>
<tr>
<td>4e</td>
<td>-1597.183329</td>
<td>-0.24455</td>
<td>-0.09189</td>
<td>3.728941</td>
</tr>
<tr>
<td>4f</td>
<td>-2453.049865</td>
<td>-0.18741</td>
<td>-0.09189</td>
<td>3.728941</td>
</tr>
<tr>
<td>4g</td>
<td>-2453.049865</td>
<td>-0.19770</td>
<td>-0.09189</td>
<td>3.728941</td>
</tr>
</tbody>
</table>

MS (EI, m/z (%)): 868 (M-, 46), 575 (39), 729 (39), 637 (46), 598 (39), 590 (39), 523 (39), 509 (46), 477 (39), 451 (46), 445 (46), 421 (39), 370 (54), 323 (46), 210 (46), 135 (46), 123 (31), 114 (46), 112 (31), 110 (39), 93 (39), 81 (54), 77 (69), 74 (39), 69 (46), 57 (100), 54 (54), 52 (39). Anal. calcld. for: C₅₂H₃₂N₆O₈: 114 (46), 112 (31), 110 (39), 93 (39), 81 (54), 77 (69), 74 (39), 69 (46), 57 (100), 54 (54), 52 (39). Anal. calcld. for: C₇₁H₇₁N₉O₉: 114 (46), 112 (31), 110 (39), 93 (39), 81 (54). Anal. calcld. for: C₇₁H₇₁N₉O₉: 114 (46), 112 (31), 110 (39), 93 (39), 81 (54).

2.2. Biological activity

2.2.1. Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce [35]. The tested organisms were sub-cultured on nutrient agar medium (Oxoid Laboratories, UK) for bacteria and Sabouraud dextrose agar (Oxoid Laboratories, UK) for fungi. Penicillin G and Streptomycin were used as a positive control for bacterial strains. Amphotericin B was used as a positive control for fungi. The plates were done in triplicate. Bacterial cultures were incubated at 37 °C for 24 h while the other fungal cultures were incubated at (25-30 °C) for 3-7 days. Antimicrobial activity was determined by measurement zone of inhibition [36].

2.2.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the samples was estimated for each of the tested organisms in triplicates. Varying concentrations of the samples (1000-0.007 μg/mL), nutrient broth was added and then a loop full of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. A tube containing broth media only was seeded with the test organisms to serve as control. Tubes containing tested organisms cultures were then incubated at 37 °C for 24 h while the other fungal cultures were incubated at (25-30 °C) for 3-7 days. The tubes were then examined for growth by observing for turbidity [37].

2.2.3. Media used

Sabouraud’s glucose agar with antibiotic: The medium used for isolation of pathogenic yeasts has the following composition (g/L): Glucose, 20; peptone, 10; agar, 25 and distilled water, 1 L, pH was adjusted at 5.4. The medium was autoclaved at 115 °C for 15 min then 0.5 g/L. Chloramphenicol was added to avoid bacterial growth [39].

Nutrient agar (NA): The medium was used to cultivate tested bacteria. It contains (g/L) Beef extract, 3; Peptone, 5 and distilled water 1 L [39].

3. Results and discussion

3.1. Chemistry

Reaction of terphthalaldehyde (2) with two mole equivalents of 3,4-dihydr(o)2(H)-naphthalen-1-one (1) in ethanoic KOH solution afforded directly (2E,2′E)-2,2′-(1,4-phenylene bis(methanylylidene))bis(3,4-dihydronaphthalen-1(2H)-one) (3) (Scheme 1). Reaction of compound 3 with nitritilines (generated in situ via triethylamine dehydro-halogenation of the corresponding hydrzonoyl halides, 4) in 1:2 molar ratio in dry benzene under reflux gave dicycloadduct compound 5, which were identified by different spectroscopic techniques as well as elemental analyses data. The IR spectra of the isolated products 5 reveal the carbonyl absorption bands in the region of 1660-1684 cm⁻¹. The appearance of a singlet signal in 1H NMR spectra at δ 5.04-5.52 ppm assignable to the pyrazole H-4, ruled out the formation of the isomeric dicycloadduct compound 6 [40-44] (Scheme 1).

On the other hand, reaction of 2,2′-(1,4-phenylene bis(methanylylidene))bis(1H-indene-1,3(2H)-dione) (8) with hydrzonoyl halides 4a-e in the 1:1 molar ratio in refluxing benzene in the presence of triethylamine as basic catalyst for 10 h, afforded only one isomeric regiosomer, the structure of which was established to be the monocy cloadduct 9 based on spectral and elemental analyses data. For example, the 1H NMR spectra of compounds 9 revealed the presence of two singlet signals at δ 5.08-5.41 ppm and 9.90-9.93 ppm assigned for the pyrazole H-4 and methylene CH protons, respectively. Repetition of the reaction of 2,2′-(1,4-phenylene bis(methanylylidene))bis(1H-indene-1,3(2H)-dione) (8) with hydrzonoyl halides 4a-e in 1:2 molar ratio in refluxing benzene for 50 h afforded two dicycloadducts compounds 10a,d and monocy clo adducts compounds 9b,c and e.

The structure of dicycloadduct compounds 10a,d was established on the bases of spectral data and elemental analyses. The 1H NMR spectra of compound 10a revealed a singlet signal at δ 5.12 ppm assigned for the pyrazole H-4 and the absence of the singlet signal of the methylene CH proton. Many attempts have been carried out for either identification or isolation of the other dicycloadduct compounds 10b,c,e isomers but they were unsuccessful, and no attempts have been made for identification of cycloadducts compounds 5, 9, 10 and their optically stereoregular configurations, using single crystal X-ray diffraction technique, but gave no satisfactory results.

3.2. Theoretical calculations of the ground state energy

The molecular geometry for all compounds under study were obtained by full geometry optimization using hybrid density functional theory B3LYP method, where (B3) [45-47] stands for Becke’s three parameter exact exchange-functional combined with gradient-corrected correlation functional of Lee, Yang and Parr (LYP) [48] by implementing 6-311G(d,p) as a basis set [49,50]. All the calculations were done using Gaussian 09 software package [51]. The optimized structures were visualized using GaussView version 5.0.9 [52].

The possible pathways products are all isomers, so the total energy can be used to investigate which pathway is more favored from the thermodynamic point of view. The energy of the reactants and products for the most stable conformer are summarized in Table 1. The energy gap ΔE is the energy difference between the energy of LUMO and energy of HOMO. The optimized geometry of compounds 3, 4a, 5a and 6a are provided in the supporting information. The energies of the products (compound 6a or 5a) are less than energies of reactants (compound 3 and 4a) with almost the same value of 80.7 Hartree this difference is the energy of the reaction.
Table 2. Antimicrobial activity expressed as inhibition diameter zones in millimeter (mm) of compounds 3, 5, 8, 9 and 10 against the pathological strains based on well diffusion as assay *.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aspergillus fumigatus</th>
<th>Syncephalastrum racemosum</th>
<th>Geotricum candidum</th>
<th>Candida albicans</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15.3±0.63</td>
<td>16.7±0.25</td>
<td>18.8±0.44</td>
<td>N.A.</td>
<td>18.3±0.19</td>
<td>19.3±0.25</td>
<td>N.A.</td>
<td>15.2±0.58</td>
</tr>
<tr>
<td>5a</td>
<td>14.3±0.58</td>
<td>15.2±0.44</td>
<td>17.3±0.58</td>
<td>N.A.</td>
<td>18.3±0.44</td>
<td>19.6±0.58</td>
<td>N.A.</td>
<td>13.4±0.44</td>
</tr>
<tr>
<td>5f</td>
<td>20.2±0.37</td>
<td>21.7±0.44</td>
<td>22.6±0.37</td>
<td>N.A.</td>
<td>20.6±0.58</td>
<td>22.1±0.25</td>
<td>N.A.</td>
<td>16.3±0.63</td>
</tr>
<tr>
<td>6</td>
<td>16.3±0.37</td>
<td>17.2±0.58</td>
<td>17.9±0.44</td>
<td>N.A.</td>
<td>18.9±0.58</td>
<td>20.2±0.63</td>
<td>N.A.</td>
<td>15.0±0.44</td>
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<tr>
<td>8</td>
<td>10.3±0.58</td>
<td>12.6±0.34</td>
<td>13.7±0.44</td>
<td>N.A.</td>
<td>11.6±0.58</td>
<td>13.0±0.67</td>
<td>N.A.</td>
<td>9.8±0.44</td>
</tr>
<tr>
<td>9a</td>
<td>12.3±0.44</td>
<td>15.9±0.58</td>
<td>16.3±0.34</td>
<td>N.A.</td>
<td>17.1±0.58</td>
<td>18.0±0.44</td>
<td>N.A.</td>
<td>24.4±0.58</td>
</tr>
<tr>
<td>10a</td>
<td>12.3±0.58</td>
<td>15.2±0.63</td>
<td>13.3±0.44</td>
<td>N.A.</td>
<td>14.2±0.25</td>
<td>15.3±0.50</td>
<td>N.A.</td>
<td>11.3±0.44</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>23.7±0.1</td>
<td>19.7±0.2</td>
<td>26.7±0.2</td>
<td>25.4±0.1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.8±0.2</td>
<td>32.4±0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.3±0.1</td>
<td>19.9±0.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* The experiment was carried out in triplicate and average zone of inhibition was calculated; (N.A. = no activity).

The stability of the products over the reactants indicates an exothermic reaction. The energy of compound 6a is slightly more stable than compound 5a by 0.71 kcal/mol. The energy difference between compound 5a and compound 6a is not vastly decisive compared to the energy of the reaction (i.e. 0.0014% of the energy of the reaction). This can be clearly seen by applying the well known Boltzman population analysis at 298.15 K for an energy difference of 0.71 kcal/mol between to singly degenerate states which results in 23.2% of the molecules will populate the upper state. The small energetic difference cannot be used to explain the stereo-selectivity of this reaction.

The better explanation for the proposed mechanism can be clearly done using a deeper look to the molecular orbital plots. Figure 1 shows the HOMO and LUMO of compounds 5a and 6a. The reaction was expected to proceed via interaction of HOMO of one reactant with LUMO of another. This treatment can give a clearer picture to which pathway the reaction will proceed by comparing the sign of the molecular orbital lobes at the site of the reaction. A similar sign indicates a possibility of bonding molecular orbital while a different signs indicates an impossible reaction or anti-bonding molecular orbital overlap. In a direct statement, the similar sign of the lobes indicates the direction of the reaction. Figure 2 shows the HOMO of compound 3 (in two different orientations to form compound 5a or 6a) and LUMO of compound 4a. The possible sites of interaction in Figure 2 of compound 3 with compound 4a are labeled with dotted arrows. The molecular orbital signs in Figure 2 show that there is only one possibility to form compound 5a (green lobes with green lobes) as it leads to constructive bonding overlap. The other pathway is not possible since color of lobes are mismatched (green lobes with red lobes) will result in destructive antibonding overlap. The molecular orbital lobes colors indicate that, there is only one possible mechanism for the interaction of the reactant to form only compound 5a. The same theoretical procedure shows that compound 5 is always more favored over compound 6 irrespective to the substituent effect.

3.3. Antimicrobial activity

In-vitro antimicrobial screening of compounds 3, 5a,b,f, 8, 9a and 10a prepared in this study was carried out using cultures of four fungal strains, including Aspergillus fumigatus (RCMB 02568), Syncephalastrum racemosum (RCMB 05922), Geotricum candidum (RCMB 05097) and Candida albicans (RCMB 05031), as well as four bacteria species, namely, Gram positive bacteria, Staphylococcus aureus (RCMB 010010) and Bacillus subtilis (RCMB 010067), Gram negative bacteria, Pseudomonas aeruginosa (RCMB 010043) and Escherichia coli (RCMB 010052). Amphotericin B as an antifungal agent, Ampicillin as an antibacterial agent for Gram positive bacteria and Gentamicin as an antibacterial agent for Gram negative bacteria was used as references to evaluate the potency of the tested compounds under the same conditions.

From the results depicted in Table 2, we found that compound 3 has moderate activity against all microorganisms used. But the pyrazoline ring found in compounds 5 dramatically increase the activity as in compound 5b which becomes more reactive than the reference drug for Syncephalastrum racemosum (RCMB 05922).
Table 3. Minimum inhibitory concentration (µg/mL) against the pathological strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fungi</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
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<td></td>
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<tr>
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<td>125</td>
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</tr>
<tr>
<td>5b</td>
<td>3.9</td>
<td>1.95</td>
<td>0.098</td>
</tr>
<tr>
<td>5f</td>
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<td>31.25</td>
<td>15.63</td>
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Figure 2. The molecular orbital plots for the HOMO of compound 3 and LUMO of compound 4a to form compound 5a in the upper and compound 6a in the lower parts.

On the other hand, the starting compound 8 is less reactive than 3, but pyrazoline ring found in compounds 9a and 10a increases slightly the activity. The minimum inhibitory concentration (µg/mL) of compound 5b showed activity more than the reference drug Amphotericin B for the fungus Syncophastrom racemosum (Table 3). From the above results, we can conclude that the pyrazoline moiety increases the activity against the microbial organisms used.

4. Conclusion

New series of compounds 5, 9 and 10 were synthesized via 1,3-dipolar cycloaddition of (2E,2′E)-2,2′-(1,4-phenylenebis(methanylethylene))bis(3,4-dihydropyridine)-1(2H)-one 3 and 2,2′-(1,4-phenylenebis(methanylethylene))bis[1H-indene-1,3(2H)-dione] 8 to a variety of nitrones intermediates. The mechanisms of the reactions studied are discussed and the structures of the products were confirmed by spectral and elemental analyses. The proposed procedure for studying the stereoselectivity of the reaction using the theoretical molecular orbital plot is highly recommended to prove the proposed mechanism theoretically. Also, molecular orbital plots for HOMO and LUMO verify our suggested mechanism and stereoselectivity of the reaction. The antimicrobial activity of the products was evaluated and promising results were obtained.

References


