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# In vitro and In silico studies on the anticancer and antimicrobial activity of Cu(II), Ni(II) and Co(II) complexes with bis (pyrazolyl) borate derivative ligand

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## Abstract

A bidentate N-donor pyrazole-based ligand abbreviated as $K[H_2B(Pz^{Me2})_2]$ and corresponding complexes with Cu(II), Ni(II) and Co(II) were synthesized and characterized, where $Pz^{Me2}$=3,5-dimethylpyrazole. The synthesized ligand and complexes were evaluated for anticancer activities against (MDA-MB-231) human breast cell line. Their Antibacterial activity against gram-positive and gram-negative bacteria was investigated. Also, their molecular docking with YmaH (PDB ID: 3HSB), ecKAS III (PDB ID: 1HNJ) protein and DNA dodecamer (PDB ID: 1BNA) as the possible targets was performed. In silico molecular docking along with the experimental MTT assay and antibacterial studies, indicated the metal complexes are more bioactive than free uncoordinated ligand and can be excellent candidates for further evaluations in the biological area.

**Keywords:** Bis(pyrazolyl)borate derivatives, Metal complexes, Anticancer activity, MDA-MB-231, Antimicrobial activity, Molecular docking.

## Introduction

Since the first reportage of poly(pyrazolyl)borates and in especially bis(pyrazolyl)borates by Trofimenko, these compounds have been developed in the inorganic coordination chemistry area and widely employed in various transition metal coordination complexes as anionic N-donor ligands.[1-5] In these type of the ligands, by controlling the number of pyrazolate rings and the substituents of different derivatives, their structure can be modified for the design and synthesis of the ligands with special properties to formation of stable complexes.[6-8] On the other hand, the excellent therapeutic properties of pyrazole-based drugs have attracted the attention of pharmaceutical chemists for the synthesis, development and investigation of various new chemotherapy drugs.[9-12] Development of the pyrazole-based coordination compounds is greatly enhanced due to the identification of their structure as anti-cancer, anti-fungal, anti-bacterial, anti-viral and anti-inflammatory agents.[13-17] On the other hand, Docking simulation can help us to understand the environment around compounds in the active site and allowing medicinal chemists to design and modify compounds. Keeping in view therapeutic and biological activities of pyrazoles based compounds and the potential of transition metals such as copper, cobalt, and nickel in the antimicrobial application area, we find it vital to join the chemistry of both moieties in designing and developing biometal compounds which could aggressively work against various bacterial species and cancer cells.[18-22] In this work, we report the synthesis and characterization of copper (II), nickel (II) and cobalt (II) complexes of dihydrobis(pyrazolyl)borate ligands. Also, antibacterial activities, MTT assay and molecular docking of these compounds were investigated.

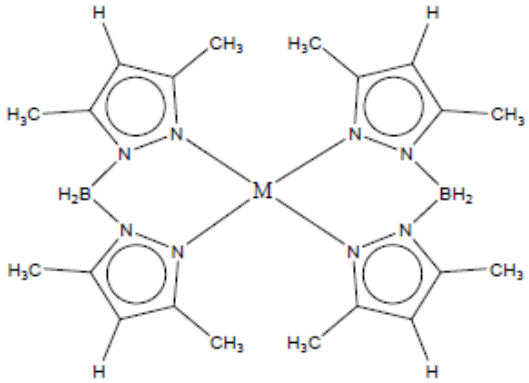
## Experimental

### *Materials and instrumentation*

All chemicals and solvents were obtained from Merck, the solvents were dried according to literature methods when anhydrous conditions were necessary. The MDA-MB-231 cell lines were obtained from Pasteur Institute (Tehran, Iran). Also, the fetal bovine serum (FBS) was bought from HyClone and the RPMI 1640 was obtained from Gibco. FT-IR spectra were recorded on a Bruker instrument in the 400-4000 $cm^{-1}$ range using KBr pellets. The melting points were determined with a Stuart Scientific SMP1 apparatus. Elemental analyses (CHN) were measured in an Elementar Vario ELIII. Disk diffusion method was applied for antibacterial investigations.

2.2. Synthesis of the ligand $K[H_2B(Pz^{Me2})_2]$ **(1)** and complexes $[M(H_2B(Pz^{Me2})_2)_2]$ (M= Cu (submitted), Ni, and Co [23]) **(2-4)**

As we previously reported, the synthesized 3, 5 dimethyl pyrazole,[21,24] (0.19 g, 2 mmol) and $KBH_4$ (0.05 g, 1 mmol) were heated to 100˚C under nitrogen atmosphere and hydrogen evolution started at this point. The temperature is raised to 130˚C gradually and was controlled evolution of hydrogen, after the production of (2 mmol) of hydrogen, the reaction was finished and the resulting white precipitate washed with the mixture of THF (20 mL) and n-hexane (10 mL). Finally, the product ligand **(1)** was dried in vacuum over $P_2O_5$. For the syntheses of complexes **(2- 4)** as described earlier,[12,21,23] the synthesized ligands **(1)** (0.48 g, 2 mmol) was dissolved in 15 ml dichloromethane and the methanolic solution of metal salts $Cu(CH_3COO)_2.H_2O$, $Ni(CH_3COO)_2.4H_2O$, $Co(CH_3COO)_2.4H_2O$ (1 mmol) were added to the ligand solution, after stirring of the reaction for overnight at room temperature. The resulting precipitate of the metal complexes was collected using vacuum filtration, and then dried in vacuo.




Scheme 1. Structure of the complexes **(2-4)**.

K[$H_2B(Pz^{Me2})_2$] (**1**): Yield: 0.16 g (67%). IR:(KBr , $cm^{-1}$): 2924-2866(w), 2435(m), 1631(w), 1585(m), 1482(w), 1422(s), 1371(w), 1111(s), 1028(m), 979(m), 948(m), 811(m), 766(m). Elem Anal. (%) Calcd for $C_{10}H_{16}BKN_4$: C, 49.59; H, 6.65; N, 23.13. Found: C, 49.55; H, 6.59; N, 23.06. m.p: 210 ºC.

[$Cu(H_2B(Pz^{Me2})_2)_2$] (**2**): Yield: 0.38 g (84%). FT-IR (KBr, $cm^{-1}$): 2958-2859(m), 2451-2276(s), 1534(s), 1495(w), 1450(m), 1416(s), 1363(m), 1321(m), 1173(s), 1110(s), 1053(m), 883(m), 801(s), 779(s), 655(m), 458(w). Elem Anal. (%) Calcd for $C_{20}H_{32}B_2CuN_8$: C, 51.14; H, 6.86; N, 23.85. Found: C, 51.22; H, 6.90; N, 23.81. m.p: >300 ºC (Dec.).

[$Ni(H_2B(Pz^{Me2})_2)_2$] **(3):** Yield: 0.33 g (72%). FT-IR (KBr, $cm^{-1}$): 2960-2860(m), 2453-2261(s), 1535(s), 1491(w), 1453(m), 1422(s), 1388(s), 1183(s), 1110(s), 1036(m), 897(m), 788(s), 622(m), 418(w). Elem Anal. (%) Calcd for $C_{20}H_{32}B_2NiN_8$: C, 51.67; H, 6.93; N, 24.10. Found: C, 51.73; H, 6.91; N, 24.05. m.p: >300 ºC (Dec.).

[$Co(H_2B(Pz^{Me2})_2)_2$] **(4)[23]:** Yield: 0.34 g (75%). FT-IR (KBr, $cm^{-1}$): 2925-2878(m), 2434-2303(m), 1653(m), 1595(s), 1533(m), 1422(m), 1306(s), 1028(s), 855(m), 778(s), 635(m),

480(w),. Elem Anal. (%) Calcd for $C_{20}H_{32}B_2CoN_8$: C, 51.65; H, 6.93; N, 24.09. Found: C, 51.70; H, 6.92; N, 23.98. m.p: >300 $^oC$ (Dec.).

***Antibacterial activity***

The synthesized compounds (**1-4**) were evaluated for their antibacterial activity against *Bacillus subtilis* (ATCC6633) and *Salmonella enterica* (ATCC14028) by the disk diffusion method. Gentamicin was used as a standard drug and Dimethyl sulfoxide (DMSO) was employed as a negative control. The bacterial cultures were developed by selective nutrient broth at 37 °C for 24 h then, were diluted to 0.5 McFarland standards. Streaking of the lawn growth in Mueller-Hinton Agar medium was performed using a cotton swab was dipped into the nutrient broth medium of the organism. This investigation was performed on Muller Hinton Agar disks with a solution of synthesized ligand and complexes (80 mM) in DMSO. The filter paper disc with a diameter of 6 mm impregnated with 10 µL of synthesized compounds solution and staid on the agar surface. After incubating the plates for 24h at 37 °C, the inhibition zone was obtained using the measurement of minimum dimensions of the zone without the bacterial growth around the disk.[21,25,26] All the tests were performed in triplicate and an average of determination was recorded.

***Cytotoxicity assay***

The synthesized N-donor ligand **(1)** and corresponding metal complexes **(2-4)** were evaluated for their cytotoxicity against human breast cell line (MDA-MB-231) by using an MTT assay. This colorimetric assay is based on the converting of the yellow (MTT) tetrazolium bromide to a purple formazan derivative mitochondrial succinate dehydrogenase in viable cells. MDA-MB-231 (human breast cell line) was prepared from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran) and was cultured in RPMI-1640 medium with 10% fetal bovine serum and were incubated in the 5% $CO_2$ and at 37 °C.[27,28] The cell lines were seeded in a 96-well

plate at a density of $1.0 \times 10^4$ cells/well and treated for 48h, with various concentrations of the synthesized investigated ligand, corresponding complexes and Paclitaxel (0.5, 1, 2, 4, 8, 16, 31, 62, 125, 250 and 500 µM). At the end of the drug treatment, 100 µL of the MTT solution (5 mg/mL in PBS) were added to each well, then incubated for 4 h.[29] The MTT formazan precipitate was dissolved in 200 µL of DMSO, and the absorbance was recorded at 595 nm by a plate reader. Untreated MDA-MB-231 cell and Paclitaxel drug were used as a negative and positive controls, respectively. This experiment was repeated at least three times independently for each condition. The $IC_{50}$ values were measured from concentration-response curves with GraphPad Prism 8 software and used to measure of cellular sensitivity to a given treatment (Table 2).

***Molecular docking***

Based on the literature and due to the fact that these proteins (YmaH, ecKAS III) and DNA have significant inhibition against the studied bacteria and investigated cell line, respectively. So these macromolecules are shown to be the possible targets for anticancer and antibacterial agents.[30-32] The molecular docking of the synthesized compounds with the YmaH (PDB ID: 3HSB), ecKAS III (PDB ID: 1HNJ) protein and DNA dodecamer (PDB ID: 1BNA) d(CpGpCpGpApApTpTpCpGpCpG) as the possible targets was performed by using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.6.[33-37] The crystal structures of the macromolecules were obtained from the Protein Data Bank website (RCSB) (http://www.rcsb.org/pdb/). The three-dimensional structures of synthesized ligand and Ni(II) complex were obtained with optimization by M06-2X method with Lanl2DZ and other base sets and the three-dimensional structures of Cu(II) complex (Submitted) and Co(II) complex were obtained from X-ray crystallography.[23] First of all, the including water molecules were deleted and the energy calculations were made by genetic algorithms. During in Silico docking, receptors were set rigid, whereas all the torsional bonds of small molecules were set free. One

hundred independent runs with the grid box 126×126×126 Å (grid spacing=0.375Å) was performed to evaluate the binding energy and other results of the inhibitors within the macromolecules.[38,39]

## Results and discussion

### *FT-IR spectral studies*

In the FT-IR spectrum of the ligand, the removal of the ν(N-H) band of pyrazole ring in the 3200 ± 50 $cm^{-1}$ range, also the appearance of the multiple peaks of ν(B-H) bond in the range of 2200- 2500 cm-$^{1}$, indicated the formation of ligand. In the FT-IR spectrum of the complexes, the bands in the region of 400-500 $cm^{-1}$ are assigned to the ν(M-N) stretching vibrations bands. The strong aliphatic bands attributed to ν(C-N) vibrations appeared in the ranges of 1321-1388 $cm^{-1}$. The shifts of other bands of the complexes in compare with the free ligand indicates coordination of the donor atoms to the metal centers.

### *Antibacterial studies*

The synthesized compounds, positive and negative controls, were screened separately to evaluate their antibacterial activity against Gram-positive (*B. subtilis*), and Gram-negative (*S. enterica*). The results were summarized in Table 1.

Table 1. Zone of inhibition of the synthesized compounds (**1-4**)

| Compounds | Zone of inhibition (mm) | |
|---|---|---|
| | *B. subtilis* | *S. enterica* |
| $K[H_2B(Pz^{Me2})2]$ **(1)** | 15.33 | 13.66 |
| $[Cu(H_2B(Pz^{Me2})_2)_2]$ **(2)** | 27.66 | 26.00 |
| $[Ni(H_2B(Pz^{Me2})_2)_2]$ **(3)** | 25.00 | 23.66 |
| $[Co(H_2B(Pz^{Me2})_2)_2]$ **(4)** | 24.33 | 22.00 |
| Gentamicin | 29.66 | 30.00 |
| DMSO | 6 (NZ) | 6 (NZ) |
| Filter paper disk | 6 (NZ) | 6 (NZ) |

NZ = no zone of inhibition; the filter disk itself is 6 mm in diameter.

The experimental result of the antibacterial investigation indicated that complexes have considerable activity compared to the free corresponding ligand. The synthesized compounds showed a zone of inhibition ranging from 15.33 to 27.66 mm against *B. subtilis* and 13.66 to 26.00 mm against *S. enterica*. Furthermore, among the metal complexes, complex $[Cu(H_2B(Pz^{Me2})_2)_2]$ (**2**) showed higher antibacterial activity than others. Positive control (Gentamicin) showed significant inhibition zones against the test microorganisms ranging from 29.66 mm to 30.00 mm against Gram-positive and Gram-negative bacteria, respectively. Dimethyl sulfoxide as a negative control revealed no inhibitory effect against any of the bacterial strains. Increasing the antimicrobial activity of coordination compounds in the comparison with the organic ligand can be explained based on chelation theory.[40,41] With the coordination of metal ions to the donor ligands, the polarity of the metal center is reduced to a greater extent due to the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Furthermore, delocalization of the π-electrons over the whole chelate ring is increased and lipophilicity of the complexes is enhanced. So the lipid solubility factor is the main factor that controls the antimicrobial activity.[42] Also, the lack of an outer membrane in the Gram-positive strains, which acts as a barrier for penetration, causes the high inhibition zone of investigated compounds against the Gram-positive strains compared with the Gram-negative bacteria.[43] Generally, metal complexes are more bioactive than free organic ligands and can be further applied in the pharmaceutical industry, as an antimicrobial agent.

***The MTT assay studies***

MTT assay was used for the determination of the cytotoxicity of the synthesized ligand and corresponding copper (II), nickel (II) and cobalt (II) complexes against human breast cancer cell line (MDA-MB-231). The obtained result was evaluated according to cell inhibition which is illustrated as $IC_{50}$ values (summarized in Table 2). The results showed that the $IC_{50}$ value of

complex [Cu($H_2B(Pz^{Me2})_2)_2$] **(2)** is much lower than free ligand **(1)** and other investigated complexes **(3,4)**, also this complex showed almost the same activity with the Paclitaxel as a drug against the investigated cell line. Generally, the coordination compounds have high cytotoxicity compared with free organic compounds and it can be explained by chelation theory which is the reason for the high cytotoxic character of the inorganic compounds.[44,45] Our experimental findings, along with molecular docking studies, suggest that the copper complex could be an excellent candidate for further anticancer studies.

Table 2. $IC_{50}$ values (µM±SD) values of the ligand **(1)**, complexes **(2-4)** and Positive control**.**

| Compound | $IC_{50}$ (µM) |
|---|---|
| K[$H_2B(Pz^{Me2})2$] **(1)** | 40.25±1.66 |
| [Cu($H_2B(Pz^{Me2})_2)_2$] **(2)** | 18.66±0.54 |
| [Ni($H_2B(Pz^{Me2})_2)_2$] **(3)** | 22.47±0.17 |
| [Co($H_2B(Pz^{Me2})_2)_2$] **(4)** | 21.39±0.85 |
| Positive control | 18.84±0.15 |

***Molecular docking analysis***

In silico Molecular docking studies were carried out by AutoDock software for a synthesized ligand and their Cu(II), Ni(II), and Co(II) complexes with the DNA and two selected proteins (YmaH and ecKAS III). The predicted binding energy and other results are summarized in Tables (3-5). According to the obtained results of these studies, the investigated receptors showed better interactions with most of the synthesized metal complexes compared to the free ligands. As can be seen in (Tables 3), the complex [Co($H_2B(Pz^{Me2})_2)_2$] **(4)** bound to DNA with the binding energy of -6.05 kcal/mol and the complex [Cu($H_2B(Pz^{Me2})_2)_2$] **(2)** bound to YmaH and ecKAS III proteins with a binding energy of -7.48 kcal/mol and -6.47 kcal/mol, respectively and showed the highest binding energy among the studied compounds. Figure 1 shows the 3D molecular docking modeling between complex [Co($H_2B(Pz^{Me2})_2)_2$] **(4)** and DNA

dodecamer. Figures 2, 3 presented the molecular docking modeling between complex $[Cu(H_2B(Pz^{Me2})_2)_2]$ **(2)** and the YmaH and ecKAS III protein, respectively. Uncoordinated free nitrogen atoms in the structure of ligand cause the formation of the hydrogen bonding with receptors, As can be seen in Figure 4, ligand $K[H_2B(Pz^{Me2})_2]$ **(1)** presented two strong hydrogen bond between the two N atom from the ligand and Asn27 group of YmaH of Bacillus substilis protein. Also, the hydrogen bond of ligand and Leu191 group of ecKAS III (PDB code: 1HNJ) protein was shown in Fig 5. Our findings from molecular docking investigation and biological assay data suggest that these synthesized complexes are the potential antibacterial and anticancer inhibitory.

Table 3. The obtained results of the docking of compounds (**1-4**) and DNA dodecamer (PDB ID: 1BNA).

| **Compound** | Binding Energy | Intermol energy | Vdw-hb-desolv-energ | Electrostatic energy | Total internal | Unbound energy | Torsional energy | Ki |
|---|---|---|---|---|---|---|---|---|
| **1** | -5.82 | -6.42 | -6.27 | -0.14 | -0.8 | -0.8 | 0.6 | 54.1 |
| **2** | -5.81 | -5.81 | -5.72 | -0.1 | 0 | 0 | 0 | 55.03 |
| **3** | -5.69 | -5.69 | -5.65 | -0.04 | 0 | 0 | 0 | 67.28 |
| **4** | -6.05 | -6.05 | -5.84 | -0.21 | 0 | 0 | 0 | 36.6 |

Table 4. The obtained results of the docking of compounds (**1-4**) and YmaH (PDB ID: 3HSB) protein.

| **Compound** | Binding Energy | Intermol energy | Vdw-hb-desolv-energ | Electrostatic energy | Total internal | Unbound energy | Torsional energy | Ki |
|---|---|---|---|---|---|---|---|---|
| **1** | -5.95 | -6.54 | -6.46 | -0.08 | -0.54 | -0.54 | 0.6 | 43.78 |
| **2** | -7.48 | -7.48 | -7.45 | -0.03 | 0 | 0 | 0 | 3.28 |
| **3** | -7.22 | -7.22 | -7.2 | -0.02 | 0 | 0 | 0 | 5.14 |
| **4** | -7.01 | -7.01 | -6.96 | -0.05 | 0 | 0 | 0 | 7.27 |

Table 5. The obtained results of the docking of compounds (**1-4**) and ecKAS III (PDB ID: 1HNJ) protein.

| **Compound** | Binding Energy | Intermol energy | Vdw-hb-desolv-energ | Electrostatic energy | Total internal | Unbound energy | Torsional energy | Ki |
|---|---|---|---|---|---|---|---|---|

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **1** | -5.64 | -6.23 | -6.17 | -0.06 | -0.66 | -0.66 | 0.6 | 73.83 |
| **2** | -6.47 | -6.47 | -6.39 | -0.08 | 0 | 0 | 0 | 18.1 |
| **3** | -6.27 | -6.27 | -6.2 | -0.07 | 0 | 0 | 0 | 25.36 |
| **4** | -5.94 | -5.94 | -5.87 | -0.07 | 0 | 0 | 0 | 44.44 |

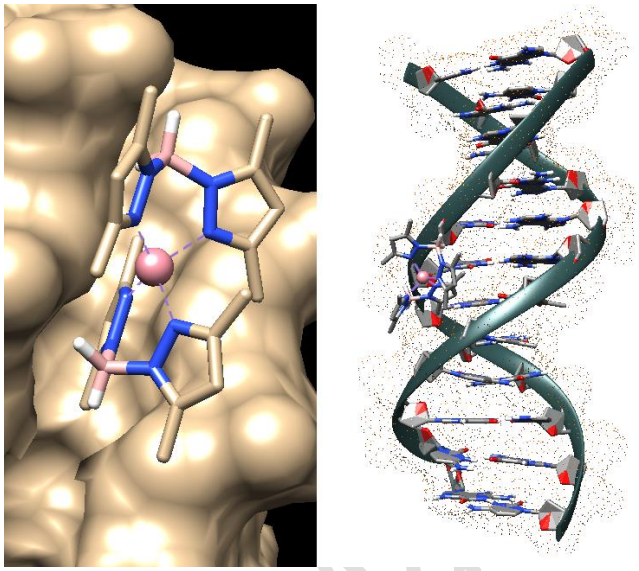


Fig 1. Molecular docking modeling between complex $[Co(H_2B(Pz^{Me2})_2)_2]$ (**4**) and DNA dodecamer.

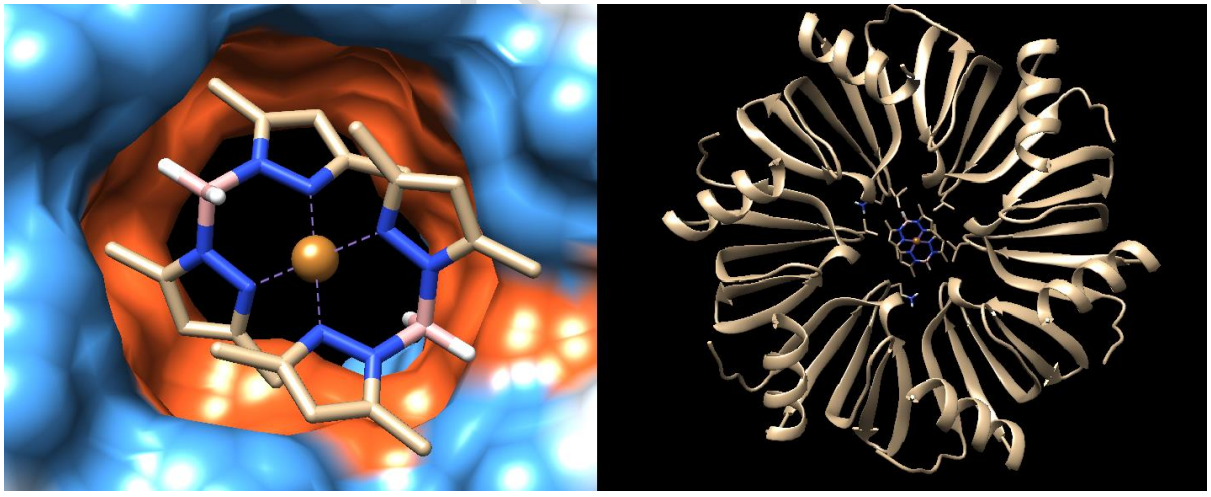


Fig 2. Molecular docking modeling between complex $[Cu(H_2B(Pz^{Me2})_2)_2]$ (**4**) and YmaH protein.

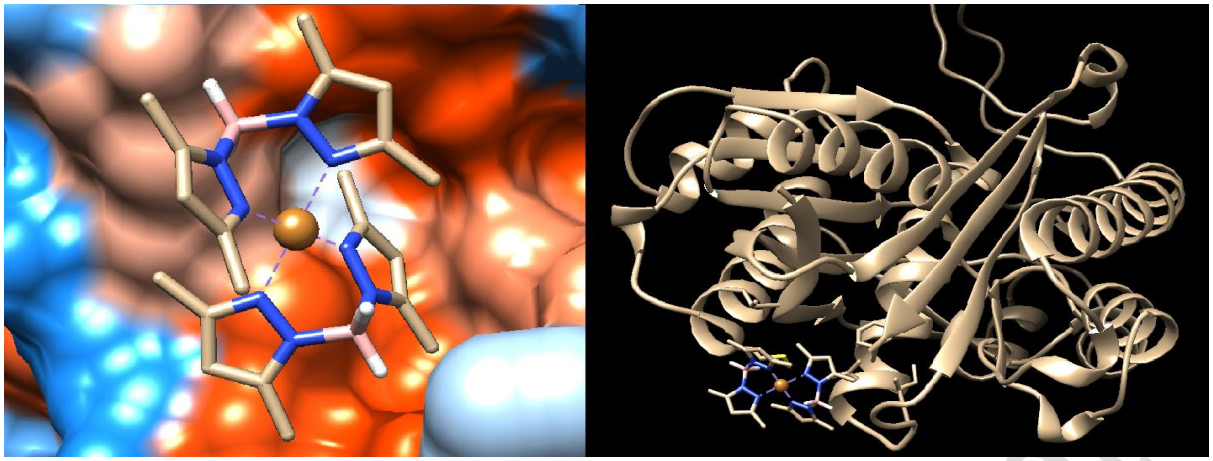


Fig 3. Molecular docking modeling between complex [Cu($H_2B(Pz^{Me2})_2)_2$] (**4**) and ecKAS III protein.

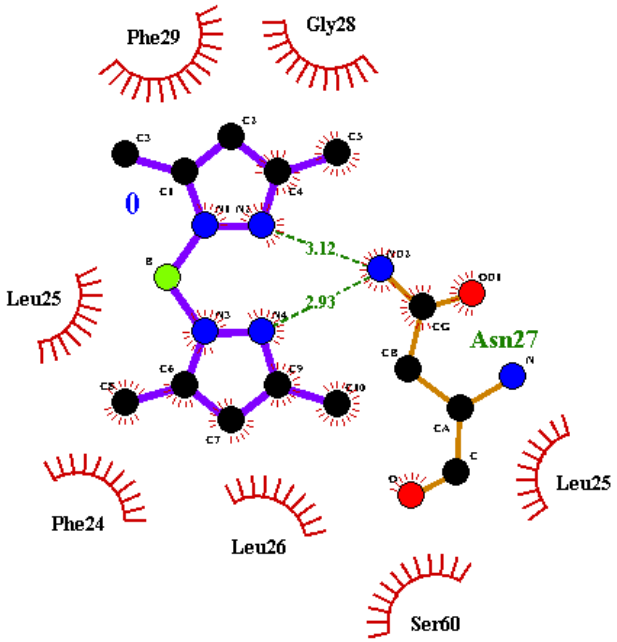



Fig 4. Binding mode of ligand K[$H_2B(Pz^{Me2})_2$] (**1**) and the YmaH protein, the H-bond (green line) is displayed as line.

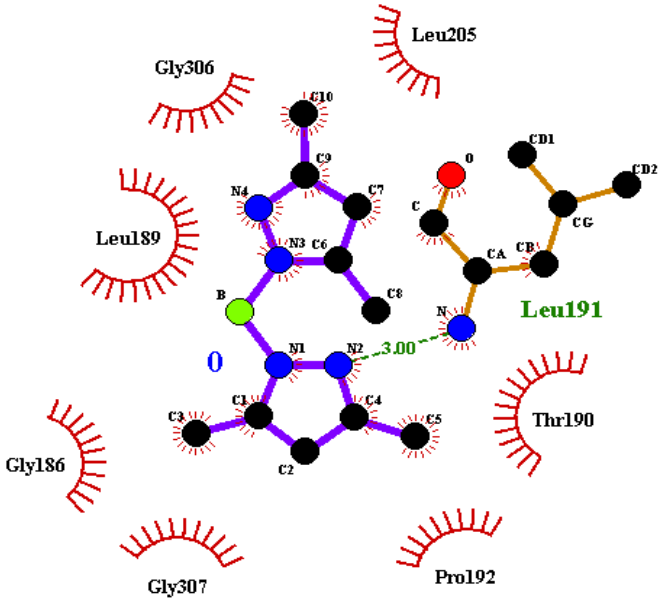



Fig 5. Binding mode of ligand K[$H_2B(Pz^{Me2})_2$] (**1**) and the ecKAS III protein, the H-bond (green line) is displayed as line.

## Conclusion

In summary, we have synthesized a type of N- donor pyrazole-based ligand and corresponding copper (II), nickel (II), and cobalt (II) complexes. The in vitro antibacterial studies against Gram-positive (*B. subtilis*), and Gram-negative (*S. enterica*) and MTT assay against MDA-MB-231 (human breast cancer) cell line carried out for the ligand as well as for the complexes. The result showed among the investigated compounds, complex [$Cu(H_2B(Pz^{Me2})_2)_2$] **(2)** indicated the highest cytotoxicity and bacterial inhibition. The molecular docking studies of synthesized compounds with the YmaH, ecKAS III protein and DNA dodecamer as the possible targets showed the complex [$Co(H_2B(Pz^{Me2})_2)_2$] **(4)** bound to DNA with the binding energy of -6.05 kcal/mol and the complex [$Cu(H_2B(Pz^{Me2})_2)_2$] **(2)** bound to YmaH and ecKAS III proteins with a binding energy of -7.48 kcal/mol and -6.47 kcal/mol, respectively and showed the highest binding energy among the studied compounds. Generally, our finding of in vitro experimental data and in silico molecular docking indicated the metal complexes

displayed good biological properties for their activity response in antibacterial and anticancer studies in compare with free ligand, which can be explained by chelation theory. Our studies results suggest that the developed pyrazolyl borate based complexes can be the good candidates for further evaluations in biological areas.

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**Author Contribution**

MG: Participated in the design of the study, analysis, revising the manuscript, interpretation of data and drafting the manuscript. BS: Participated in the design of the study, analysis, revising the manuscript, interpretation of data, drafting the manuscript and final approval of the version to be published. OM: Participated in the design of the study, revising the manuscript, interpretation of data and final approval of the version to be published. EM: Participated in the design of the study, analysis, revising the manuscript, interpretation of data and final approval of the version to be published.

**Conflict of Interest**

The authors have declared no conflicts of interest.

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