

SEARCH FOR CONFORMATION OF THIOSEMICARBAZONE REAGENTS AND THEIR COMPLEXES WITH METALS BY USING MONTE CARLO AND DOCKING SIMULATION

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(Received: 05 September 2019; Accepted: 08 July 2020)

Abstract. In this study, the conformation of ML_2 complexes of new thiosemicarbazone reagents with metal cations Cd^{2+} , Ni^{2+} , Cu^{2+} , Hg^{2+} , Pb^{2+} , Mn^{2+} , and Zn^{2+} is investigated. The methods include MM+ and PM3 calculations with the Monte Carlo techniques using the Metropolis algorithm in the temperature range of 298–473 K. The initial selection conformation was carried out randomly after 15 repeated conformations, and 30 conformations were rejected. The conformations were chosen to change by changing the torsional-dihedral angles at the position of the metal cation associated with the donor atoms N and S of the thiosemicarbazone reagents. This was performed by randomly changing the dihedral angles to create new structures, and then the energy values of these angles were minimized with the PM3 and MM+ calculation. The lowest suitable energies were accumulated, while high- or duplicate-energy structures were discarded. The docking method was also employed to screen the most suitable metal-thiosemicarbazone complexes that bind to the active site on the SARS-CoV-2 protein. The docking method enabled us to choose the molecular conformation of the most significant Cd^{2+} -thiosemicarbazone complex.

Keywords: thiosemicarbazone reagent, Monte Carlo simulation, PM3 and MM+ calculation, SARS-CoV-2

1 Introduction

The generation of new starting conformations for energy minimization uses the random variation of dihedral angles [1]. Rotation is used for acyclic bond dihedral angles. In a ring, dihedral angles rotate due to the “torsional flexing” motion of Kolossváry and Guida [2], which effectively leads to new ring conformations, while avoiding large atomic displacements that can decrease the efficiency of optimization.

Nowadays, thiosemicarbazone reagents are members of the organic compounds that have numerous applications in medicine and analytical chemistry [3, 4]. Some thiosemicarbazone reagents are known to possess anticancer bioactivity [4, 5], but they can form stable complexes with metal ions. This property of thiosemicarbazone reagents can be used in analyzing environmental and food samples. For these reasons, the search for a stable conformation of thiosemicarbazone complexes has so far not been conducted systematically. The conformational search of cyclic molecules plays a

central role in studying molecular structure and dynamics [6, 7].

In this work, we report the use of an approach for the conformational search by combining the molecular-mechanical methods with the Monte Carlo search technique for thiosemicarbazone ligands and metalthiosemicarbazone complexes. Then, the energy minima of the metal-thiosemicarbazone complexes were determined with the semi-empirical calculations PM3 SCF and MM+ molecular mechanics to estimate the energy. The stability of the conformations were assessed from the stable molecular energy according to the possible global and local minima of the complexes. The conformation of complexes can be considered to perform docking into the active site of SARS-CoV-2 protein. The complexes of thiosemicarbazone with metal ions were also used to investigate the inhibition ability for SARS-CoV-2 by docking to the active site on the SARS-CoV-2 protein.

2 Computational details

2.1 Ligand conformation

The conformational search for reagents and thiosemicarbazone complexes aims to identify the conformations of a molecular system with low potential energy surface [2, 7]. Along with determining the global minimum of potential energy surfaces, it is important to identify all minima that generate heat and thus affect the macroscopically observed characteristics of the system. The 3D molecular structure of thiosemicarbazone reagent (E)-2-((6-bromo-9-ethyl-9H-carbazol-3-yl)methylene) hydrazine-1-carbothioamide and complexes of this reagent with a metal cation is constructed by the use of the Hyperchem program [5] (Fig. 1).

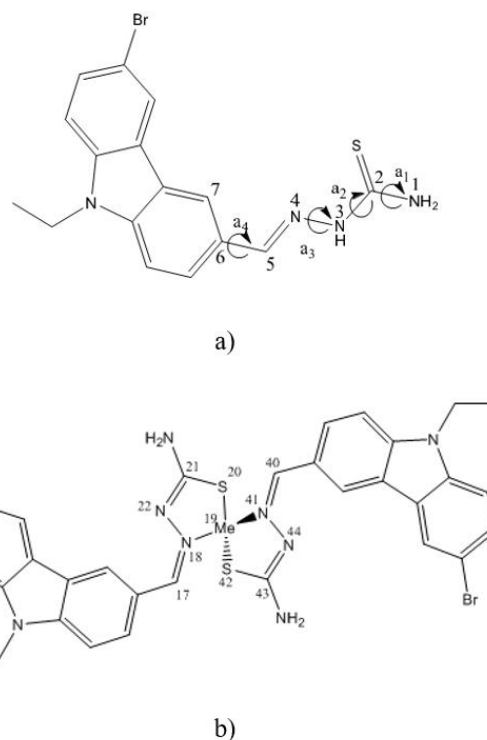


Fig. 1. Molecular skeleton: a) thiosemicarbazone ligand; b) complex of thiosemicarbazone with metal ion Me. Rotatable bonds and dihedral angles during the conformation search

The thiosemicarbazone reagent and its complexes were optimized by using the MM+ molecular mechanics force field and the semi-empirical PM3 [5] method. These structures are inputted for the conformational search performed on this package [6]. The thiosemicarbazone reagent has four rotatable single bonds, depicted with the arrows and the rotation angles (Fig. 1). These angles are abbreviated as follows: a1: HN₁-C₂-N₃, a2: N₁-C₂-N₃-N₄, a3: C₂-N₃-N₄-C₅, and a4: N₄-C₅-C₆-C₇. These angles varied from 0 to 360 degrees with 10-degree increments by using the semi-empirical method PM3 SCF [5] to determine the structures with minimum energy.

The dynamic properties of complexes can be implemented with the Monte Carlo methods, which randomly move to a new conformation. The conformation with lower energy or very close in energy is accepted; otherwise, an entirely new

conformation is generated. This continues until a set of low-energy conformers is generated [6]. The torsional angles of the complexes, considered for metal bonds and the donor atoms N and S, are abbreviated as follows: t1: C₁₇-N₁₈-Me₁₉-N₄₁, t2: C₂₁-S₂₀-Me₁₉-N₁₈, t3: C₄₃-S₄₂-Me₁₉-N₄₁, and t4: N₁₈-Me₁₉-N₄₁-N₄₄. The number of simultaneous variations varies from 1 to 8. The ranges of acyclic torsion variables vary from ±60 to 180. The ranges for ring torsion flexing vary from ±30 to 120. The geometries are optimized by using the molecular mechanics method MM+ [5] to determine the structures with minimum energy at the RMS gradient 0.05 and the maximum cycles 3000. For these systems, a search by systematic variation of all conformational parameters is possible [6].

2.2 Calculation methods

The discovery of a conformational search is to perform the conversion of the parameters affecting potential energy. Because the molecular flexibility is usually a result of the rotation of the dihedral and torsional angles, many different conformations are possible. To reduce the computational cost, high energy structures are removed, or molecular energy can be minimized by using rapid tests to separate atoms. The atoms cannot bond and bond as disadvantages. This case can lead to a molecular structure the bonding length of which is too long or too short. In this case, the molecular energy that can be accepted by the Metropolis algorithm is in the temperature range from 298 to 473 K, after 15 processes and 30 processes. The process of finding information about a shape can be carried out in four steps [5, 6, 8]:

- Making an initial structure selection;
- Changing the original structure by changing the geometric parameters;
- Optimizing the modified structure into a structure with minimized energy;

- Comparing conformation with what was found earlier and accepting if it is unique and if the energy meets the criteria.

In step 1, the original molecular structure can be kept intact and unchanged throughout the search process. In the search for validation with the Monte Carlo technique, the random walk option for this step usually requires an increase in temperature from 298 to 473 K during the acceptance test. This may increase the probability of accepting high energy molecular structures that can overcome potential barriers. The temperature adjustment is usually performed after repeatedly finding the same type of duplicated structure or can continuously reject new processes based on the criteria of the Metropolis algorithm.

In step 2, the last acceptable choice of conformation is often called the random walk phase of the Monte Carlo search process. This process is based on the observation that suitable low energy tends to be similar, so the molecule starting from an accepted structure should tend to be retained for searching in the low energy region of the potential surface. The low-energy region meets requirements. The search process should not be stuck in the local low energy area. This was introduced in the Monte Carlo algorithm with many minimum molecular energy levels (MCMM) [6, 8].

In step 3, the structural optimization of the molecule used to find local minimums on the potential surface starts from the molecular structure in step 2. In this step, choosing the molecular optimizer will little impact on the information about the structure. Even so, the use of rapid convergent optimization may fall to a local minimum energy level that cannot overcome the barrier on the potential energy surface. The second important point to note is that local minima cannot be ignored, but this is also often an important feature of efficiency optimization.

In step 4, both conformation and energy tests are used as criteria for accepting new conformations. The molecular structure is consistent with the inversion centers. This can be arbitrarily removed with energy meeting the criteria.

2.3 Docking methods

In recent years, molecular docking has become an increasingly important tool for screening and searching for the mechanism of action of drugs [9]. In this study, we briefly present the thiosemicarbazone complex docking on the protein-active site of SARS-CoV-2. Relevant theoretical foundations include sampling algorithms and docking score calculations. We used the flexible receptor molecular docking method for the protein of SARS-CoV-2, especially the methods that include the flexibility of receptors, which will be a challenge for docking methods. An approach based on the Monte Carlo algorithm (MC) [7, 10] has recently been developed as a potential solution to flexible receptor docking problems.

3 Results and discussion

3.1 Conformation of thiosemicarbazone ligand

We carried out further calculations for rotational barriers of flexible bonds. The lowest-energy conformer is generated. The conformational analysis is performed by rotating the corresponding dihedral angles, following the

procedure described in the method section. The energies needed to transpose the barrier between the maximal and minimal energy conformers vary considerably, depending on the dihedral angle (Table 1). The different variation of energetic barriers depends on each dihedral angle.

The absolute difference between the minimum and maximum energy of the energetic barrier dihedral a_1 and a_2 suggests there are numerous low-energy and stable conformations that populate the potential energy surface. The rotation of dihedral a_1 and a_2 leads to the energy needed to pass the barrier between the maximal and minimal energies. This energy is equal to 18.5434 kcal·mol⁻¹ for angle a_1 and 20.5512 kcal·mol⁻¹ for angle a_2 . The lowest-energy conformation corresponds to the torsion angle equal to 180°, and the two highest values of torsional angles are equal to 90° and 270° (Fig. 2a).

The rotation dihedral a_3 can generate an energetic barrier of 20.220 kcal·mol⁻¹. The two lowest-energy conformations have torsional angles of 60° and 300°. From the rotation of the dihedral angle, a_4 , two highest-energy conformations are recognized when the dihedral angle, a_4 , varies from 70 to 100° and from 260 to 290°, respectively. The lowest-energy conformation is found to be corresponding to dihedral a_4 180°. In this case, it can generate an energy barrier of 3.115 kcal·mol⁻¹ (Fig. 2b). Therefore, the reaction activity of the thiosemicarbazone reagent can depend on one of the conformations corresponding to the lowest energy.

Table 1. Rotational energy barriers corresponding to each dihedral

	Dihedral angle							
	Energy kcal·mol ⁻¹	a_1	Energy kcal·mol ⁻¹	a_2	Energy kcal·mol ⁻¹	a_3	Energy kcal·mol ⁻¹	a_4
Min	-3878.806	180	-3889.930	180	-3880.095	60	-3878.805	180
Max	-3860.263	90	-3869.379	80	-3859.875	360	-3875.691	280

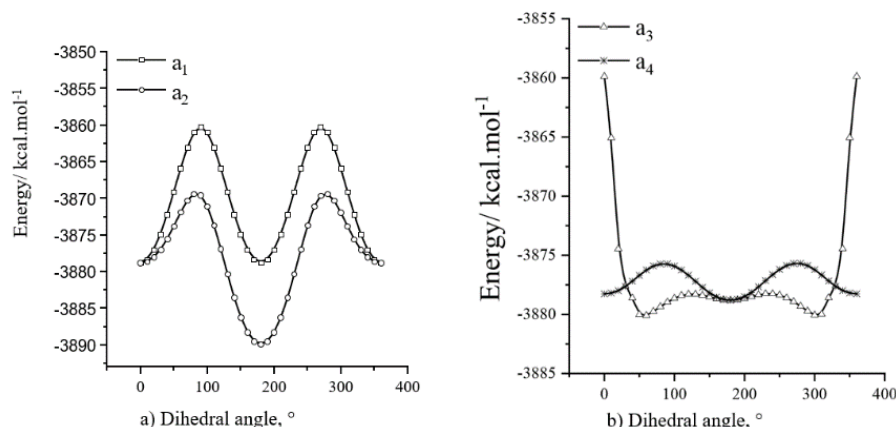


Fig. 2. Rotational energy barriers for dihedral angles for new thiosemicarbazone reagent: a) dihedral angles a_1 H-N1-C2-N3 and a_2 : N1-C2-N3-N4; b) dihedral angles a_3 : C2-N3-N4-C5 and a_4 : N4C5-C6-C7

3.2 Complexes conformation

For metal-thiosemicarbazone complexes, the torsional flexing (FLEX) t1: C17-N18-Me19-N41, t2: C21-S20-Me19-N18, t3: C43-S42-Me19-N41, and t4: N18-Me19-N41-N44 (Fig. 1b) of complexes for metal ions Cd²⁺, Ni²⁺, Cu²⁺, Hg²⁺, Pb²⁺, Mn²⁺, and Zn²⁺ are considered as local torsional rotation about a ring bond that protects the atomic position of most of the ring atoms. The conformational search implemented by a set of low-energy conformations is generated several times. For these complexes, the ring bonds between the metal ion and N and between S and the metal ion are protected, resulting in two thiosemicarbazone fragments. All other associations to N rotate in one direction about the N–Me bond, and all other associations to the metal ion rotate in the opposite direction about the S–Me bond. For the process of conformational search, both geometric and energetic tests are used as criteria for accepting new conformations. The conformations with chiral centers that have inverted may optionally be discarded. The results of the conformational search for each complex are presented in Table 2.

For implementing the conformational search, we first explore the optimum ring bonds that could be simultaneously submitted to torsional flexing during each Monte Carlo step for metal-thiosemicarbazone complexes. We have also inquired the optimum mutation of torsional angles. 1000 Monte Carlo iterations are utilized to apply to various flexing angles. We investigate the effect of simultaneously flexing four ring bonds (this is found randomly) with the randomly torsional angle alterations selected from four different angular ranges. The investigation to be used for checking chirality and determining duplication is specified. The energetic inspection may be either a cutoff relative to the best energy or a Metropolis criterion. The temperature adjustment from 298 to 473 K may be used with the Metropolis criterion. The conformational geometries of low-energy complexes Cu²⁺L₂, Cd²⁺L₂, Ni²⁺L₂, Mn²⁺L₂, Zn²⁺L₂, Pb²⁺L₂, and Hg²⁺L₂, corresponding to their quantity are found by searching procedure (Fig. 3.)

Table 2. Searched conformations of complexes at temperature range 298 to 473 K

Complex	Conformations	Lowest found	Highest kept	Accept rate	Iterations	Torsion tests
Cd ²⁺ L ₂	15	65.2903	163.8078	0.234	1246	3
Cu ²⁺ L ₂	23	48.4294	50.2096	0.253	1170	2

Complex	Conformations	Lowest found	Highest kept	Accept rate	Iterations	Torsion tests
Hg ²⁺ L ₂	23	103.5891	118.4575	0.180	1207	1
Mn ²⁺ L ₂	14	83.5703	97.2144	0.155	1233	0
Ni ²⁺ L ₂	20	82.2663	95.8973	0.131	1206	1
Pb ²⁺ L ₂	26	102.7820	117.8339	0.242	1248	1
Zn ²⁺ L ₂	11	88.8526	102.0090	0.170	1182	0

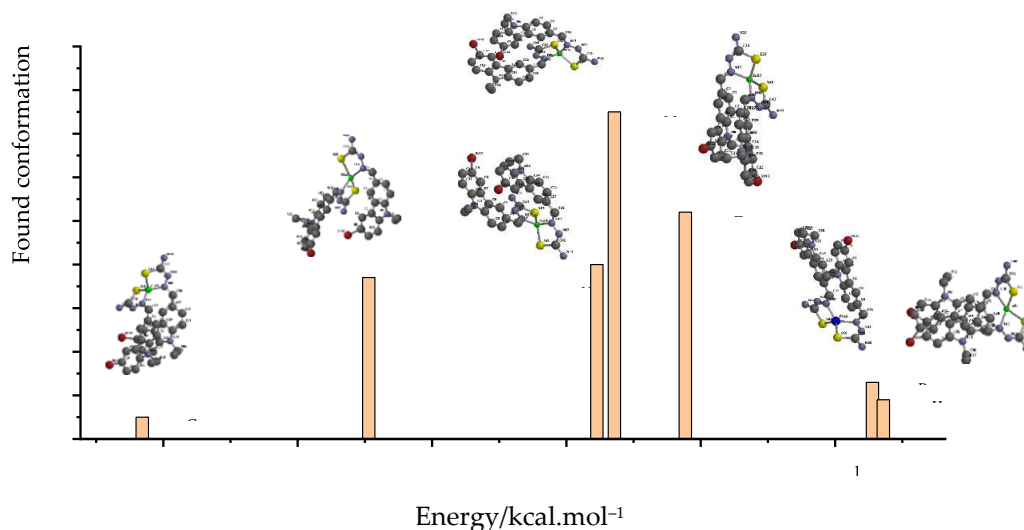


Fig. 3. Lowest-energy conformations of complexes for four torsional angles C₁₇-N₁₈-Me₁₉-N₄₁, t₂: C₂₁-S₂₀-Me₁₉-N₁₈, t₃: C₄₃-S₄₂-Me₁₉-N₄₁, t₄: N₁₈-Me₁₉-N₄₁-N₄₄

The considered results show that conformational searches based on the torsional flexing procedure are successful, concerning the finding potency for low-energy conformations of the thiosemicarbazone reagent and metal-thiosemicarbazone complexes.

3.3 Docking metal-thiosemicarbazone

As we all know coronavirus 2019 (SARS-CoV-2) causes acute respiratory syndrome and has spread rapidly worldwide. Due to its highly contagious properties discovered by Munster et al. [11] and Zhu et al. [12], there have been many studies focusing on SARS-CoV-2 inhibitor design by docking simulation [13-15]. SARS-CoV-2 is 82% homologous with the SARS-CoV genome sequence as reported by Chan et al. [16]; SARS-CoV-2 is 82% homologous to the SARS-CoV genome sequence

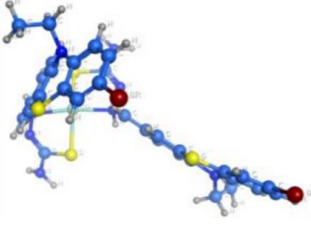
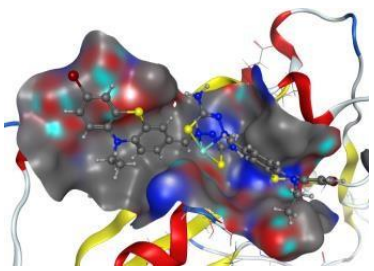
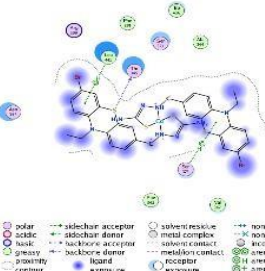
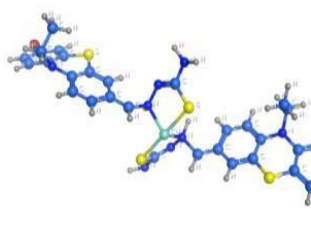
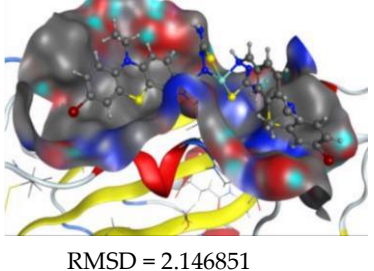
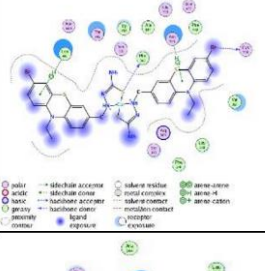
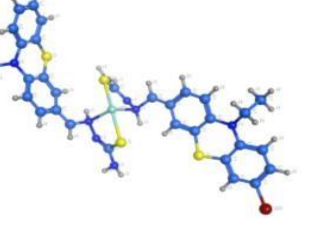
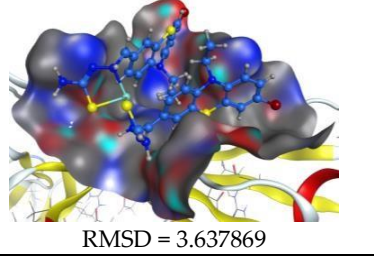
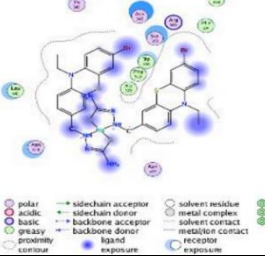
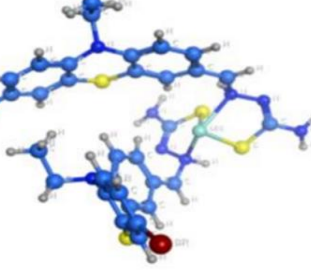
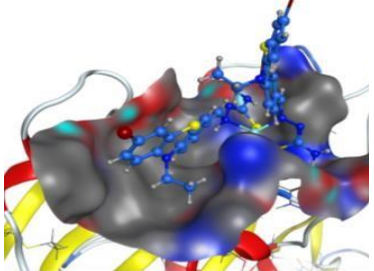
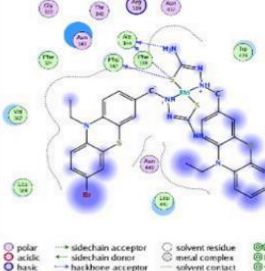
reported by Chan et al. [16]; SARS-CoV-2 patients often exhibit mild symptoms, such as fever, cough, muscle aches, and fatigue, and often have a good prognosis. Especially in Vietnam, at present, SARS-CoV-2 still has many potential risks because there is no specific medicine for it. Numerous studies in Vietnam perform docking simulations for drugs for SARS-CoV-2. The inhibiting mechanism of the SARS-CoV-2 virus is still unknown. The whole world is searching for SARS-CoV-2 medicine. One of the SARS-CoV-2 treatment regimens is the use of drugs being treated for HIV patients. Another regimen is using chloroquine, and the world is now following this direction.

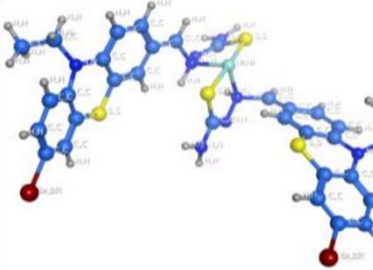
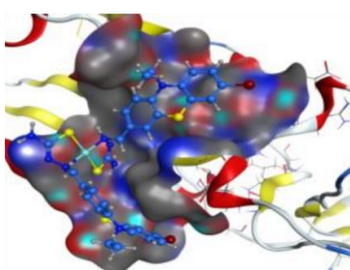
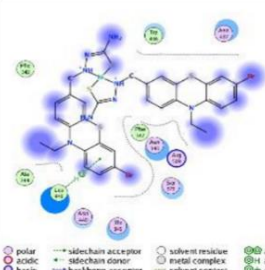
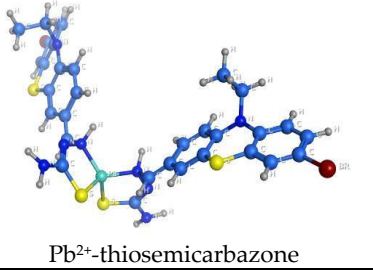
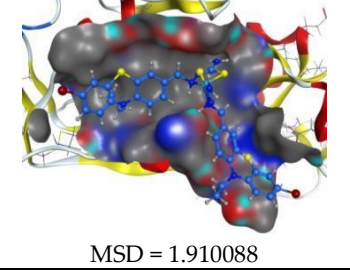
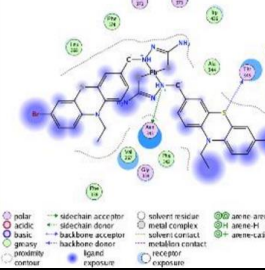
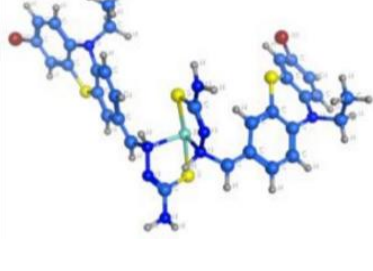
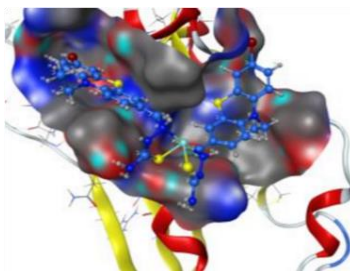
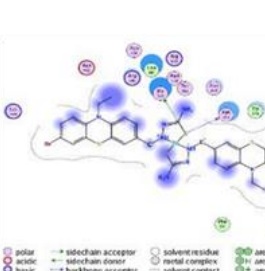
These drugs are all in the process of evaluating and researching treatment for SARS-CoV-2 patients in Vietnam. There are currently also drugs with antimicrobial, antifungal, and antiviral

activity under review. The complexes of thiosemicarbazone with metal ions fall in this category. The new complexes of thiosemicarbazone with Cd^{2+} , Cu^{2+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , Pd^{2+} , and Zn^{2+} , designed and synthesized by Quang et al. [17, 18] are used to perform docking on the active site of the SARS-CoV-2 protein. We predicted the protein spatial structure of SARS-

CoV-2 from a database. We determined the active sites of the SARS-CoV-2 protein to bind the metal-thiosemicarbazone complexes to the protein. There are also new docking results for the complex conformations, which have been searched above. The docking results of metal-thiosemicarbazone complexes are shown in Table 3.

Table 3. Comparison of docking results of metal-thiosemicarbazone complexes on the active sites of the SARS-CoV-2 protein

Complex conformation	Docking model	Ligand interaction	$E_{\text{Conf}}/\text{kcal mol}^{-1}$
 Cd^{2+} -thiosemicarbazone	 RMSD = 3.113713		-446.098
 Cu^{2+} -thiosemicarbazone	 RMSD = 2.146851		-412.729
 Hg^{2+} -thiosemicarbazone	 RMSD = 3.637869		-394.412
 Mn^{2+} -thiosemicarbazone	 RMSD = 8.45424		-527.493

Complex conformation	Docking model	Ligand interaction	$E_{Conf}/kcal$ mol^{-1}
 <p>Ni²⁺-thiosemicarbazone</p>	 <p>RMSD = 6.960862</p>		-435.941
 <p>Pb²⁺-thiosemicarbazone</p>	 <p>MSD = 1.910088</p>		-255.289
 <p>Zn²⁺-thiosemicarbazone</p>	 <p>RMSD = 5.646092</p>		-488.024

During docking a ligand into the active site of SARS-CoV-2 protein, we found that the total binding energy between metal-thiosemicarbazone complexes and amino acids surrounded the active site follows the order $Mn^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Cu^{2+} > Hg^{2+} > Pb^{2+}$. In addition, we can also use the value of root-mean-square deviation (RMSD) to assess the binding ability of ligands at the active site. The RMSD value is frequently used to measure the differences between the values predicted by a model, and determine the binding capacity of thiosemicarbazone complexes with metal ions, which follows the order $Pb^{2+} > Cu^{2+} > Cd^{2+} > Hg^{2+} > Zn^{2+} > Ni^{2+} > Mn^{2+}$. For the metal-thiosemicarbazone complexes, we found that the Cd^{2+} -thiosemicarbazone complex is the most important in binding to the active and potential

sustainable site. It satisfies two important factors: the conformational energy of the system and the binding to amino acids. Thus this complex can to bind well with amino acids.

4 Conclusion

The conformational searches for the thiosemicarbazone reagent and its complexes suggest that to assure that a full search of the conformational space available to multiacyclic complexes is implemented one could utilize more than one procedure of conformational search. On the other hand, torsional flexing could appear as the unavoidable choice for conformational searches on multiacyclic substances, such as metalthiosemicarbazone complexes. These are the

conformations of new thiosemicarbazone ligands with metal ions synthesized by us, and they are also new conformations of complexes.

The use of Monte Carlo simulation and docking simulation techniques can successfully screen the significant conformations to dock a metal-thiosemicarbazone complex ligand to an active site of the SARS-CoV-2 protein. We have determined the conformational structure of the complex Cd^{2+} -thiosemicarbazone, which is the most suitable complex for inhibiting the SARS-CoV-2 protein to attach angiotensin-converting enzyme (ACE).

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