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A THEORETICAL STRUCTURAL STUDY OF ISONIAZID COMPLEXES WITH THIOTRIAZOLINE

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*A combined molecular mechanics and quantum chemistry modeling was performed for investigation of the structure and relavive stability of two- and three-component complexes formed by the antituberculosis agent isoniazid with morpholinium (3-methyl-1,2,4-triazol-5-yl)thioacetate (MTTA). The possible interactions between the molecules were identified by molecular mechanics calculations, and the stability of the complexes was calculated by the density functional method B97-D/6-311G**, with accounting for the solvent effects in the SMD continuum model. The calculations showed that stable complexes of isoniazid with this thiatriazoline are possible both in the gas phase (ΔG^{298} = -13.6 kcal/mol) and in aqueous solution (ΔG^{298} = -7.6 kcal/mol). The formation of two-component complexes between isoniazid and MTTA without involving morpholine is considerably less favored (ΔG^{298} = -6.6 kcal/mol in the gas phase and ΔG^{298} = -2.6 kcal/mol in solution). Thus, morpholine may be considered as a component facilitating the formation of isoniazid complexes with MTTA.*

Keywords: isoniazid, thiotriazoline, conformational search, molecular complexes, quantum-chemical calculations.

Tuberculosis is a very common human infectious disease. It has been estimated that one third of the human population has been infected, and every second brings a new infection [1-4]. The therapeutic agents for the treatment of tuberculosis include derivatives of isonicotinic acid hydrazide, antibiotics, and modern combined drug regimens [1-2].

The most potent drug against *Mycobacterium tuberculosis* is the hydrazide of isonicotinic acid, isoniazid (**1**), which serves as one of the most common first-line antituberculosis medications. In addition to the positive pharmacotherapeutic effect, isoniazid also has toxic effects on liver, central and peripheral nervous system, cardiac and systemic hemodynamics, therefore the prevention of toxicity due to isoniazid still is an important field of study. The inclusion of antioxidants in combined drug regimens for the treatment of

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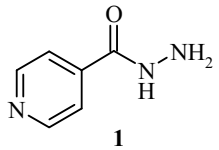
infectious diseases is a very promising approach. There is a recent trend in the pharmaceutical industry towards the development of novel drug combinations based on active ingredients and antioxidants that have compatible physicochemical and pharmacological characteristics. Such a combination was proposed for the mitigation of isoniazid toxicity, by adding the antioxidant thiotriazoline (**2**), which is morpholinium (3-methyl-1,2,4-triazol-5-yl)thioacetate (MTTA) [5].



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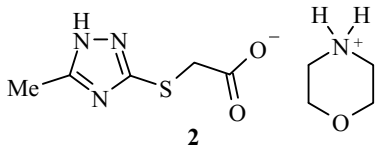

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The combined use of isoniazid and thiotriazoline would benefit from the preparation of sufficiently stable intermolecular complexes of these compounds. For this reason, we were interested in structural and thermodynamic study of complexes formed by isoniazid, MTTA, and morpholine, both in the gas phase and in aqueous solutions.

Theoretical modeling of the energy and structural characteristics for possible complexes of isoniazid and thiatriazoline is a nontrivial task due to a variety of reasons. These molecules have several proton donating and accepting sites that may or may not be involved in hydrogen bonding, depending on the mutual orientation and conformation of the molecules. The molecules of MTTA are conformationally labile. It was shown that this molecule may exist in several almost isoenergetic conformations [6]. Therefore the identification of possible molecular complexes required the application of methods that guarantee the analysis of all possible molecular conformations and mutual arrangements of molecules in the complexes. This task is conceptually similar to molecular docking – the search for the optimal interaction of a given molecule with a target, such as a macromolecule or its fragment, for example, the active site of a protein.

Bearing in mind the difficulty of finding all possible interactions in such a three-component system, this problem can be solved with reasonable computational resources only at the molecular mechanics (MM) level. On the other hand, the MM methods cannot ensure sufficiently reliable energies. Therefore the results of molecular mechanics modeling need to be refined through quantum-chemical calculations. For this purpose, we applied the density functional B97D [7], which includes empirical dispersion correction and is one of the most appropriate functionals for the estimation of intermolecular interaction energy [8]. The 6-311G** basis set was selected [9]. The solvation effects were modeled by the semiempirical continuum method SMD, which is one of the most accurate continuum model, in particular for the calculation of solvation energies of ionic compounds [10]. Water was used as model solvent. All quantum-chemical calculations were performed with the Gaussian 09 software package [11].

Thus, we used the following scheme for identifying the most stable complexes of isoniazid with MTTA and morpholine:

– the candidate complexes with diverse molecular orientation and conformations were found by genetic conformational search using the MMFF94 force field realized in the OpenBabel program [12]. A total of approximately 10000 complexes were calculated for each system, which guarantees the complete coverage of variation space;

– the 100 energetically most favorable complexes were selected from all complexes identified in the previous step, and preliminary geometry optimization was performed with the (SMD-)B97D/6-31G* method;

– the final optimization of 10 most energetically favored complexes identified in the previous step was performed with (SMD-)B97D/6-311G**, followed by Hessian calculation at the same level of theory.

It should be noted that the expansion of the basis set from 6-31G* to 6-311G** had almost no impact on the structures of the complexes, all hydrogen bonds and the mutual orientation of the molecules were conserved. The stability sequence of the complexes was also practically unaffected.

Vibration frequencies were calculated in the harmonic approximation and the Gibbs free energy was calculated at 298 K in the rigid rotor approximation. The energy of complex formation was calculated as a difference between the free energy of the complex and the free energy sum of its isolated constituents.

The calculated structure, energy values, and characteristics of the intramolecular hydrogen bonds of the four most stable two- and three-component complexes in the gas phase and in aqueous solution are presented as Supplementary information.

The results of quantum-chemical structural and energy calculations of molecular complexes between isoniazid, MTTA, and morpholine are given in Tables 1 and 2, while the structure of isolated molecules and the numbering of atoms are shown in Figure 1. All the most stable complexes identified contain multiple hydrogen bonds between the components, apparently providing the main mode of intermolecular bonding. The mismatch between the number of proton donating and accepting functional groups in the complex forming molecules allow no possibility of simultaneous involvement of all proton donors in hydrogen bonding. The geometric limitations and the conformational flexibility of the molecules, especially MTTA, caused a range of various orientations between the various components in the identified complexes, with diverse intermolecular interactions. Furthermore, in all of the identified most stable three-component complexes each of the components are hydrogen bonded to the other two components. Also, there are no intramolecular hydrogen bonds found in any of the most stable complexes.

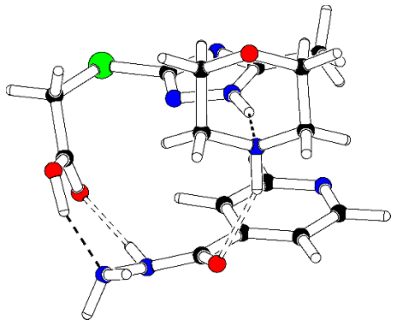
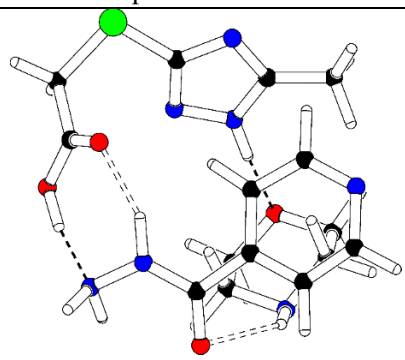
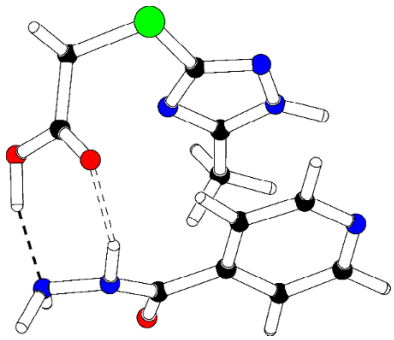
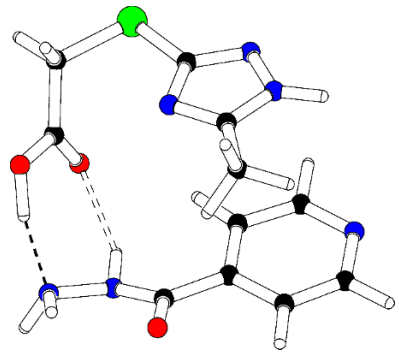
It should be pointed out that the molecule of MTTA in the complexes is in "orthogonal" conformation, where the CH_2–COOH bond was practically perpendicular to the triazole ring plane. It was shown earlier that such a conformation is not energetically favored for a free MTTA molecule, but exists in crystalline state and in aqueous solutions [6].

Quantum-chemical calculations indicate that the formation of a three-component complex **N3-g** consisting of isoniazid, MTTA, and morpholine is energetically favored in the gas phase (Table 1). The carboxyl group of MTTA and the hydrazine group of isoniazid in this complex are linked by two strong hydrogen bonds O(2)–H···N(1) and N(2)–H···O(3), which may be characterized as resonance-enhanced hydrogen bonds [13]. The protonated triazole ring atom forms a hydrogen bond with the morpholine molecule, which in turn is linked by a hydrogen bond to the carbonyl group of MTTA. The complex **N3-w** is the most stable in aqueous medium and also has a similar structure (Table 2). Remarkably, much stronger hydrogen bonds O(2)–H···N(1) (1.64-1.67 Å) are present in solution, compared to the gas phase (1.78-1.81 Å), while the bonds N(7)–H···O(1) were weakened (2.09-2.21 and 2.35-2.41 Å in the gas phase and solution, respectively). The calculated energy of complex formation in aqueous solution is significantly lower than in gas phase. Nevertheless, the energy of formation was found to be negative in both cases, thus the complexes are thermodynamically stable.

The most stable two-component complexes of isoniazid with MTTA also feature intermolecular hydrogen bonds between the carboxyl and hydrazine groups, the O(2)–H···N(1) bonds also were very strong (H···N 1.64-1.67 Å). However, the triazole ring forms no hydrogen bonds. Apparently this is due to the additional steric and conformational restraints to their formation. The energy of formation for the two-component complexes in gas phase is considerably lower than that of the three-component complexes, while in solution these values were approximately equal.

Since MTTA and morpholine are an acid and a base, a proton transfer is possible between the carboxyl group of MTTA and the nitrogen atom of morpholine, leading to salt formation. According to quantum-chemical calculations, such an ionization process and the separation of ion pair are not favorable neither in gas phase (ΔG^{298} = 124.4 kcal/mol) nor in aqueous solution (ΔG^{298} = 2.1 kcal/mol). Despite this, the formation of bonded ion pairs may be assumed, in which protons are transferred within the molecular complexes. For this reason, we also performed a search for corresponding stable complexes and calculated the energy of formation for such complexes, against the reference of separate, neutral component molecules.

TABLE 1. The Structures of the Most Stable Isoniazid Complexes with MTTA and Morpholine in the Gas Phase and in Aqueous Solution, the Free Energy of Complex Formation, and the Characteristics of the Intermolecular Hydrogen Bonds D–H···A According to (SMD-)B97D/6-311G** Calculations

Gas phase			Aqueous solution		
					
N3-g			**N3-w**		
ΔG_{form}^{298} = -13.4 kcal/mol			ΔG_{form}^{298} = -2.2 kcal/mol		
Bond	H···A distance, Å	D–H···A angle, º	Bond	H···A distance, Å	D–H···A angle, º
N(2)–H···O(3)	1.87	149	N(2)–H···O(3)	1.91	150
N(6)–H···N(7)	1.76	164	N(6)–H···O(4)	1.77	175
N(7)–H···O(1)	2.09	151	N(7)–H···O(1)	2.41	137
O(2)–H···N(1)	1.78	162	O(2)–H···N(1)	1.67	169
					
N2-g			**N2-w**		
ΔG_{form}^{298} = -6.6 kcal/mol			ΔG_{form}^{298} = -2.6 kcal/mol		
Bond	H···A distance, Å	D–H···A angle, º	Bond	H···A distance, Å	D–H···A angle, º
N(2)–H···O(3)	1.88	149	N(2)–H···O(3)	2.06	141
O(2)–H···N(1)	1.76	162	O(2)–H···N(1)	1.66	165

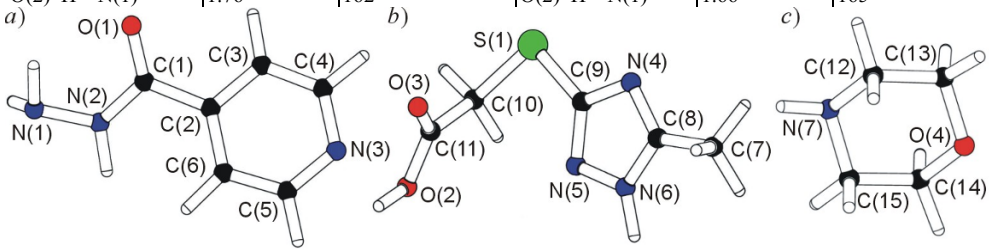
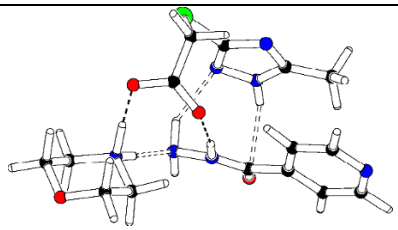
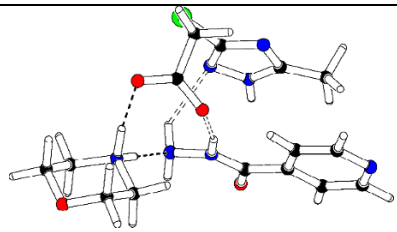
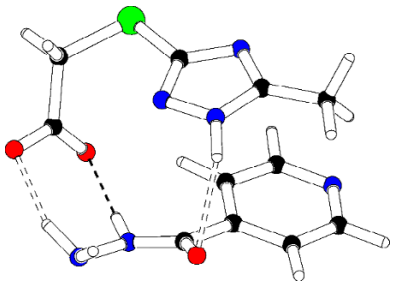
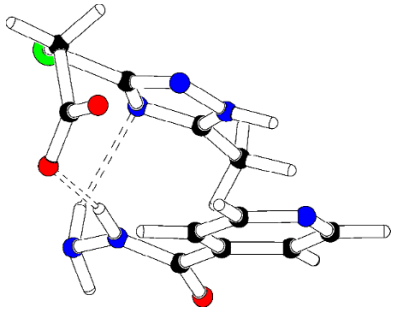


Fig. 1. The molecular structures of *a*) isoniazid, *b*) MTTA, and *c*) morpholine with the numbering of atoms.

The most stable three-component ionic complexes have similar structures in the gas phase and in solution (Table 2). We should note the formation of very short hydrogen bond between the positively charged NH_2 group of morpholine and the negatively charged carboxyl group in the complex **Z3-g**, with the H···O distance equal to 1.44 Å. Such a short distance between a proton and acceptor may indicate the tendency for a reverse proton transfer, and thus low stability of such a complex [14]. At the same time, the energy of formation for the ionic complex **Z3-g** is similar to that for the neutral complex **N3-g**. The stability of the ionic three-component complex **Z3-w** in aqueous solution was considerably higher than the stability of the neutral complex **N3-w**, where the N(7)–H···O(3) hydrogen bond is not shortened. This indicates that the aqueous medium facilitates the formation of ionic complexes between isoniazid and thiotriazoline.

TABLE 2. The Structures of the Most Stable Ionic Complexes of Isoniazid with MTTA and Morpholine in the Gas Phase and in Aqueous Solution, the Free Energy of Complex Formation, and the Characteristics of the Intermolecular Hydrogen Bonds D–H···A According to (SMD-)B97D/6-311G** Calculations

Gas phase			Aqueous solution		
					
Z3-g			**Z3-w**		
ΔG_{form}^{298} = -13.6 kcal/mol			ΔG_{form}^{298} = -7.6 kcal/mol		
Bond	H···A distance, Å	D–H···A angle, º	Bond	H···A distance, Å	D–H···A angle, º
N(1)–H···N(5)	2.13	143	N(1)–H···N(5)	2.31	137
N(2)–H···O(2)	1.76	165	N(2)–H···O(2)	1.85	165
N(6)–H···O(1)	2.45	119	N(7)–H···N(1)	1.79	172
N(7)–H···N(1)	1.85	168	N(7)–H···O(3)	1.72	169
N(7)–H···O(3)	1.44	175			
					
Z2-g			**Z2-w**		
ΔG_{form}^{298} = 94.3 kcal/mol			ΔG_{form}^{298} = -0.2 kcal/mol		
Bond	H···A distance, Å	D–H···A angle, º	Bond	H···A distance, Å	D–H···A angle, º
N(1)–H···O(3)	1.92	159	N(1)–H···N(4)	2.35	139
N(2)–H···O(2)	1.78	152	N(2)–H···O(3)	1.84	167
N(6)–H···O(1)	2.26	138			

The formation of two-component ionic complexes is highly unfavorable in the gas phase, with the energy of formation up to +94 kcal/mol, which could be explained by the high energy of breaking the ionic bonds in the gas phase. The formation of such complexes in aqueous solutions is also energetically unfavorable, because the calculated free energy of formation is close to zero.

Thus, the results of quantum-chemical calculations for the complexes of isoniazid with morpholinium (3-methyl-1,2,4-triazol-5-yl)thioacetate indicate the possibility of forming such complexes both in gas phase, as well as in aqueous solutions. The complexes formed in the gas phase consist of neutral molecules, with the energy of formation equal to -13.6 kcal/mol, while the formation of complexes in aqueous solutions involve a proton transfer from the carboxyl group of MTTA to the nitrogen atom of morpholine. The energy of formation for such ionic complexes is up to -7.6 kcal/mol. The formation of two-component complexes between isoniazid and MTTA without the involvement of morpholine is considerably less favored both in gas phase and in solution phase. Therefore, morpholine can be considered as a component that facilitates the complex formation between isoniazid and MTTA.

REFERENCES

1. B. V. Noreiko, *Novosti Meditsini i Farmacii*, **19**, 261 (2008).
2. *Global tuberculosis control: WHO Report 2002*, Geneva (2002), p. 295.
3. S.-W. Lee, L. S.-C. Chung, H.-H. Huang, T.-Y. Chuang, Y.-H. Liou, and L. S.-H. Wu, *Int. J. Tuberc. Lung Dis.*, **14**, 622 (2010).
4. D. García de Viedma, M. Marín, S. Hernangómez, M. Díaz, M. J. Ruiz Serrano, L. Alcalá, and E. Bouza, *Arch. Intern. Med.*, **162**, 1873 (2002).
5. I. A. Mazur, L. I. Kucherenko, T. Yu. Vinnichenko, A. I. Grinashchuk, E. E. Kalashnikova, N. A. Avramenko, and O. V. Khromyleva, RU Pat. 2501797.
6. R. I. Zubatyuk, S. V. Shishkina, L. I. Kucherenko, I. A. Mazur, and O. V. Shishkin, *Struct. Chem.*, **19**, 407 (2008).
7. S. Grimme, J. Antony, S. Ehrlich, and H. Krieg, *J. Chem. Phys.*, **132**, 154104 (2010).
8. J. Antony and S. Grimme, *Phys. Chem. Chem. Phys.*, **8**, 5287 (2006).
9. R. Krishnan, J. S. Binkley, R. Seeger, and J. A. Pople, *J. Chem. Phys.*, **72**, 650 (1980).
10. A. V. Marenich, C. J. Cramer, and D. G. Truhlar, *J. Phys. Chem. B*, **113**, 6378 (2009).
11. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, *Gaussian 09*, *Revision B.01* (2009).
12. N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, *J. Cheminf.*, **3**, 33 (2011).
13. V. Bertolasi, P. Gilli, V. Ferretti, and G. Gilli, *J. Chem. Soc.*, *Perkin Trans. 2*, 945 (1997).
14. P. Gilli, V. Bertolasi, L. Pretto, and G. Gilli, *J. Mol. Struct.*, **790**, 40 (2006).