

Conformational Analysis of Some Hydrazinecarbothioamide Derivatives with In Vitro Antimicrobial Activity.

2. A Case Study for N-Allyl-2-(5-Fluoro-2-Oxo-1-(Piperidin-1-Ylmethyl)Indolin-3-Ylidene)Hydrazinecarbothioamide

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Abstract: In the present study a conformational analysis has been performed for N-allyl-2-(5-fluoro-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene) hydrazinecarbothioamide in order to determine the possible global and local minima on the potential energy surface (PES). The analysis has been carried out using molecular mechanics (AMBER96) and semiempirical (AM1 and PM3) methods and the results were compared to identify the best possible approach to be used for studying the conformational space of the title compound. Also, the ligand's reorganization capacity and its conformational space have been investigated. The conformational analysis of the title compound has been carried out with Conformational Search Module implemented in HyperChem 7.5 software package. The initial geometry of the molecule was the standard one given by the same software package and optimized with AMBER96 force field and AM1 semiempirical method. This starting geometry has been further employed in subsequent calculations.

Keywords: hydrazinecarbothioamide derivatives; conformational analysis; semiempirical methods; AM1; PM3

1. Introduction

Nowadays, tuberculosis is still a high mortality contagious disease worldwide [1,2]. Several drugs are known, e.g. rifampicin, pyrazinamide, isoniazid, and ethambutol, for the treatment of tuberculosis infections, but poor compliance to the treatment schema and/or high mobility of the population has led to the appearance of multidrug-resistant *Mycobacterium tuberculosis* strains [3]. For these reasons, high interest is dedicated to the development of new and more potent agents with anti-tuberculosis activity.

In the present work, a conformational analysis study using semi-empirical methods implemented in HyperChem 7.5 [4] followed by energy minimization of the conformers at semi-empirical level with Mopac2009 [5] was performed. The results were analyzed in order to identify the best approach for the determination of possible global and local minima for the title compound.

2. Methods

The 3D structure of N-allyl-2-(5-fluoro-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene) hydrazinecarbothioamide was constructed with the Build module implemented in HyperChem 7.5 software package and subsequently, optimized using AMBER96 force field [6] and semiempirical method AM1 [7] implemented in the above mentioned software package. This structure was

used as input for the conformational analysis carried out with Conformational Search module implemented in the same software package. The title compound has 7 rotatable bonds, marked by arrows in Fig 1 and their corresponding torsion angles are abbreviated as follows: a1: N14-C15-C16-C17, a2: C12-N14-C15-C16, a3: N11-C12-N14-C15, a4: N10-N11-C12-N14, a5: C3-N10-N11-C12, a6: C4-N5-C19-C20, and a7: N5-C19-C20-C21.

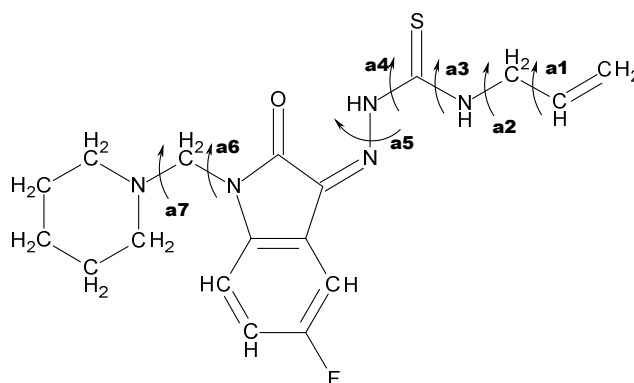


Figure 1. Structure of N-allyl-2-(5-fluoro-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene) hydrazinecarbothioamide and the numbering of heavy atoms. Bonds rotated during the conformational analysis are marked with arrows and the corresponding dihedrals are also noted

These dihedrals were varied from 0 to 360 with 30-degree steps using the AMBER96 force field and

semiempirical methods AM1 and PM3 [8], in order to find structures near possible global minima. For each approach, the maximum number of conformers to be generated was set to 1000, an energy window of 20 kcal/mol above the global minimum was used and 100 conformers which met this energy criterion were kept. Then, the AMBER conformers were energy minimized using semi-empirical methods AM1 and PM3, implemented in HyperChem software package, for up to 1000 cycles and the cutoff value for RMS gradient was set to 0.01 kcal/mol. The sets of conformations generated with AM1 and PM3 were checked and those having unreliable geometries, in terms of bond lengths, angles and dihedrals, were discarded. Subsequently, the selected conformations were fully optimized using the semi-empirical methods AM1 and PM3 implemented in MOPAC 2009 software package. In order to evaluate compound's potential to easily undergo conformational changes, energy barriers for each rotatable bond were evaluated scanning the corresponding dihedrals from 0 to 360 degrees in 5-degree steps. For each generated conformation single point calculations were performed using semi-empirical method AM1 implemented in HyperChem 7.5 and the results were thoroughly analyzed.

3. Results and Discussion

It is important to mention here that the conformational analysis has been carried out for all rotatable bonds of the ligand, in order to correctly evaluate the conformational space of the title compound, the dihedrals which cause important steric effects and to obtain

information about putative minima obtained through the conformational analysis of those dihedrals.

The first issue which has been addressed in this work is the choice of method to perform the conformational analysis. For this reason different approaches and methods have been used and their outcome is compared to identify the best suited one for our case. Shortly, the input 3D conformation of the title compound was subjected to conformational analysis performed with HyperChem 7.5 with the variation of the seven dihedrals, depicted in Fig.1. The search was performed by three different approaches: using the AMBER96 force field (1) and semi-empirical methods: AM1 (2) and PM3 (3). The AMBER96 force field has been chosen to perform the molecular modeling because it is well parameterized for a wide variety of bioactive molecules and it is fast achieving a good speed/performance ratio. Subsequently, the top 100 conformations obtained from the conformational search performed with AMBER96 force field were subjected to geometry optimization by AM1 (4) and PM3 (5) methods implemented in HyperChem. The set of conformers generated by semi-empirical methods AM1 and PM3 implemented in HyperChem 7.5 were subjected to further optimization with the same methods: AM1 (6) and PM3 (7) implemented in MOPAC2009. Finally, vibration analysis of all MOPAC conformations was carried out in order to identify and discard possible wrong conformations.

The heat of formation for the lowest energy conformers and energetic barriers computed from each approach as the difference between the heat of formation of the highest energy conformer and the lowest energy conformer are presented in Table 1.

TABLE 1. The energetic barriers computed from each approach

Approach	ΔE^* (kcal/mol)	Distinct conformers	ΔH_f (kcal/mol)	Observations
2	3.4904	70	38.3938	Conformers generated and optimized with AM1 using HyperChem
3	25.8772	90	54.1011	Conformers generated and optimized with PM3 using HyperChem
4	17.3417	85	39.2434	Conformers generated with Amber96 force field and optimized with AM1 using HyperChem
5	17.1733	95	53.7725	Conformers generated with Amber96 force field and optimized with PM3 using HyperChem
6	3.4885	64	38.6817	Conformers generated with AM1 using HyperChem and optimized with MOPAC/AM1
7	8.2552	90	22.91	Conformers generated with PM3 using HyperChem and optimized with MOPAC/PM3

* ΔE (kcal/mol) – the energy differences computed as the difference between the ΔH_f (heat of formation) of the highest-energy conformer and the lowest-energy one.

A pattern has been noticed when AM1 method was used for calculations. Namely, the lowest energy conformers generated by the use of approaches which employed this method: (2), (4) and (6) have similar ΔH_f (the differences are less than 1 kcal/mol) while the energetic differences calculated between the lowest and highest energy conformer are nearly identical for (2) and (6), but significantly different for (4). In the last case (4), AMBER96 force field was used in the conformational search step. This behavior shows that AMBER96 allows a

larger number of conformers to be generated, but the optimization process leads to the same low energy conformation. Also, the geometries of the lowest energy conformers attained via (2), (4), and (6) were compared, superimposed and the root mean square deviation (RMSD) was calculated. As shown in Fig. 2, the geometries are identical for conformers generated via (2) and (6), with a RMSD of 0.1 Å and slightly different when superimposing the geometries (2) – (4) and (4) – (6) respectively, with a value for RMSD around 1.2 Å (for both cases). Also, for all

the low-energy geometries some common features have been noticed, namely a planar geometry for the central core of the molecule which comprises the aromatic fused ring and hydrazinecarbothioamide moiety, and Z stereochemistry of the conformers with respect to the double bond between C3 and N10. These geometrical features allow the formation of an intramolecular H-bond, depicted with a black dashed-line in Fig. 2, between O18 and H atom belonging to N11, adding additional stability to the system.

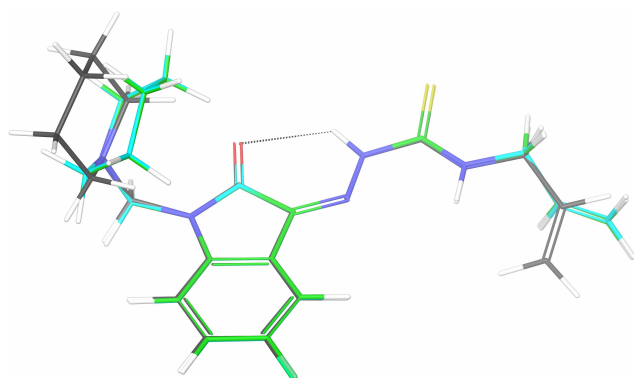


Figure 2. The superimposition of the lowest energy conformers obtained using AM1 in different approaches. Conformational search performed with: AMBER96 and minimized with AM1 (carbons colored in gray) (4), AM1 (carbons are colored in green) (2), and AM1 and fully minimized with MOPAC at AM1 semi-empirical method (carbons are colored in cyan) (6)

When semi-empirical PM3 method was used in different approaches: (3), (5) and (7), a pattern could not be distinguished and more interestingly, a correlation could not be observed between the approach used and the observed results, similarly to the previous case. However, the lowest energy conformers generated via (3) and (5) approaches show similar magnitudes for the heat of formation, the difference is very small, of 0.3286 kcal/mol, although the energetic differences computed from these approaches are significantly different (Table 1). When conformational search was performed with PM3 method implemented in HyperChem 7.5 and the obtained conformations were subsequently optimized at semi-empirical level PM3 with Mopac 2009, approach (7), the lowest value for the heat of formation has been noticed (Table 1). The superimposition of the lowest energy conformers obtained from (3), (5) and (7) is presented in Fig. 3.

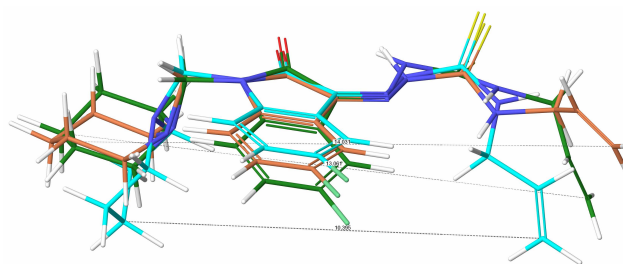


Figure 3. The superimposition of the lowest energy conformers obtained using PM3 in different approaches. Conformational search performed with: AMBER96 followed by the minimization with HyperChem/PM3 (carbons colored in cyan) (5), PM3 (carbons colored in green) (3), and PM3 followed by full minimization with MOPAC at PM3 semi-empirical method (carbons colored in orange) (7)

A thoroughly analysis of the conformations generated and/or optimized with PM3 method has led to the following observations: (i) the geometries of the lowest energy conformers are considerably different (Fig. 2), their superimposition giving the following RMSD values: 1.114 Å for (3) – (7), 1.594 Å for (5) – (7) and 1.794 Å for (3) – (5), respectively; (ii) when conformational search is performed by approach (5) a folded geometry of the lowest energy conformer is generated, the distance between the two most widely separated atoms is 10.395 Å, while for approach (7) this distance is almost 4 Å higher, giving a less folded conformation (Fig. 2), with a more relaxed geometry having reduced steric repulsions. This could be a plausible explanation for the smaller magnitude of ΔH_f of the last mentioned approach (Table 1); (iii) the pyramidalization of all nitrogen atoms within molecule was observed for all conformers, regarding of the variant of the PM3 approach used. Due to this phenomenon, the intramolecular H-bond is not formed leading to a destabilization of the structure and higher values for heat of formation.

Taking into account the observations presented above, together with the results collected and analyzed, we have decided to perform further calculations, specifically rotational barriers for all flexible bonds, using the lowest energy conformer generated via approach (6). The analysis was performed by rotating the corresponding dihedrals, following the procedure already described in the Methods section. As can be seen from Table 2, our results show that the energies needed to transpose the barrier between the maximal and minimal energy conformers vary considerably depending on the dihedral. The magnitude of the energetic barriers ranges from 1.834 kcal/mol for dihedral a1 up to 1941.973 kcal/mol in the case of dihedral a5.

TABLE 2. The rotational energy barriers corresponding to each dihedral

	a1		a2		a3		a4		a5		a6		a7	
	ΔH_f (kcal* mol^{-1})/Angle($^\circ$)													
MIN	38.394	230	38.392	240	38.396	175	38.408	360	38.396	180	38.408	275	38.403	65
MAX	40.228	350	52.643	350	57.756	0	51.414	250	1980.369	0	661.341	160	47.297	340
DIF	1.834		14.250		19.360		13.006		1941.973		622.933		8.894	

The low magnitude of the energetic barrier for dihedral a1 suggests there are numerous low-energy and stable conformations which populate the potential energy surface, as can be seen from Chart 1. The transition from one conformation to another is possible for this dihedral because, in some cases, the energy needed to surmount the barrier is smaller than 0.5 kcal/mol.

As can be seen from Table 2, when rotating the dihedral a2 the energy needed to pass the barrier between the maximal and minimal energies is about 14.25 kcal/mol. a2 has the energy minima at 240°, but there are numerous conformations located in an energy window less than 1kcal/mol when this dihedral ranges from 120° to 280° (Chart 2). These observations suggest that, in the bioactive conformation, the rotation of this dihedral is possible and it is likely for the title compound to pass from one low-energy conformation to another one, with respect to the rotatable bond corresponding to dihedral a2.

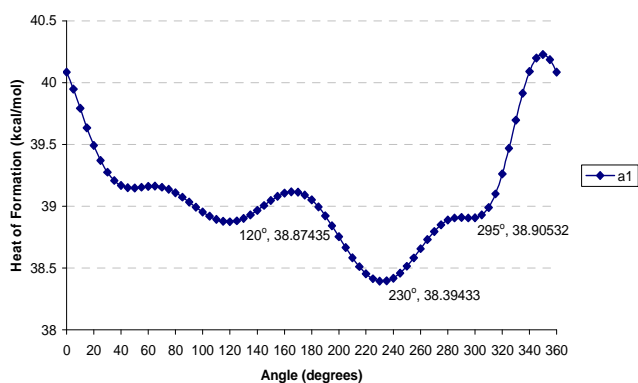


Chart 1. Rotational energy barriers for dihedral a1

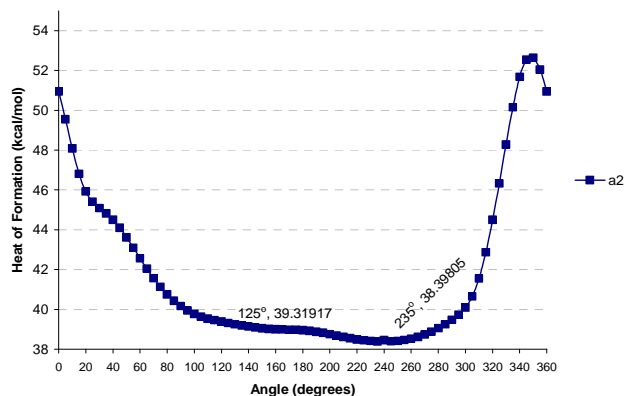


Chart 2. Rotational energy barriers for dihedral a2

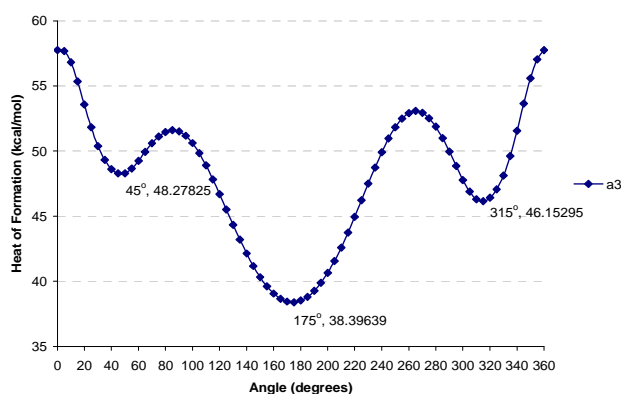


Chart 3. Rotational energy barriers for dihedral a3

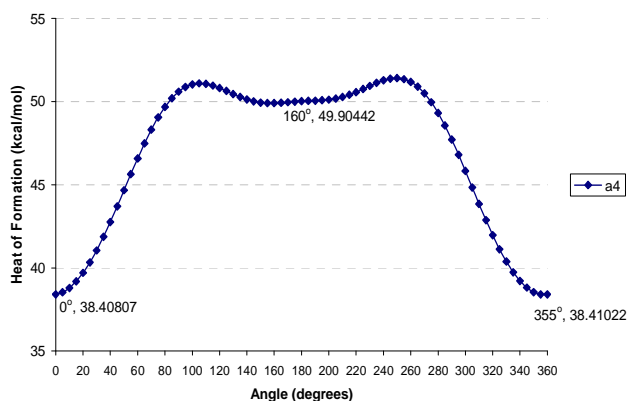


Chart 4. Rotational energy barriers for dihedral a4

When rotating dihedral a5 the highest magnitude of the energetic barrier, compared to the other dihedrals, has been achieved with a value of 1941 kcal/mol (Chart 5). Based on the values for the heat of formation, three groups of conformers have been identified, corresponding to a local minimum between 175° and 185°, two intermediate conformational states corresponding to the following ranges: 70° - 90° and 265° - 285°, and two very high energy

The rotation of dihedral a3 leads to an energetic barrier of 19.36kcal/mol, the lowest energy conformation corresponding to a value of torsion equal to 175° (Chart 3). Two intermediate conformational states have been noticed when dihedral a3 varies from 310 to 320° and 40 to 55°, respectively.

The results obtained from the rotation of dihedral a4 are depicted in Chart 4. In this case, two low energy conformations have been found corresponding to 0° and 360°, respectively. Also, two high-energy conformations have been detected around 100° -110° and 240° -260°, but the rotational barrier to overcome, in order to pass from a local minima conformation to a maximal energy one, is around 13 kcal/mol. It is difficult to surmount a barrier having this order of magnitude and probably, in the bioactive conformation, this angle can have one of the values corresponding to the low-energy conformations.

states when dihedral a5 varies between 0° up to 45° and 315° to 360°. Even though two intermediate conformational states have been identified, the energetic barriers needed to be surmounted in order to get from a local minimum conformation to a transitional one are high, 14 kcal/mol. The heat of formation for the most stable conformation is estimated at 38.396 kcal/mol and dihedral a5 is valued at 180°, corresponding to a planar geometry of the C-N-N-C

bond. In this geometry the hydrazine hydrogen atom is properly oriented to form an intramolecular H-bond with the carbonylic moiety which brings stability to the system. When dihedral a5 is rotated and the corresponding bond is out of the planar orientation this H atom can not form the favorable intramolecular interaction anymore. On the other

hand, the rotation of this dihedral leads to steric hindrances between the central core of the molecule and the side chain of N11. These facts could explain the high magnitude of the rotational barriers and it suggests that for the bioactive conformation this could be the only possible value of dihedral a5.

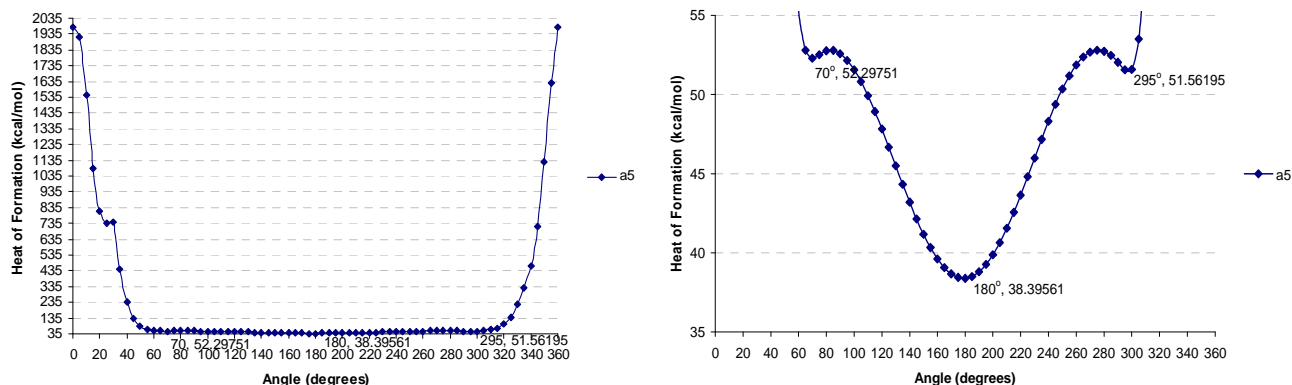


Chart 5. Rotational energy barriers for dihedral a5 (left), and a detail (right)

Also, in the case of dihedral a6 high energy rotational barriers have been noticed, as can be seen from Table 2 and Chart 6, similar to dihedral a5. Conversely, two sets of low energy conformations were found, one between 65° and 120° and the other one between 220° and 300°. A couple of intermediate states have been identified but the energetic barriers between the low-energy conformations and the intermediate ones have magnitudes in the order of tens, suggesting that it is impossible to surmount these barriers in order to pass from a low energy conformation belonging to the first set to a low-energy one from the second set. Overall, in the bioactive conformation, dihedral a6 is

limited to the values mentioned above and conformational reorganization of the ligand in the protein binding site with respect to dihedral a6 is highly improbable due to steric hindrance.

Finally, the analysis of dihedral a7 variation (Chart 7) has shown the major rotational energy barrier is about 8.9 kcal/mol. This result suggests it is difficult to surmount the barrier and pass through a low-energy conformation located between 60° and 90° to an intermediate conformation or to a high-energy conformer, located between 330° and 340°.

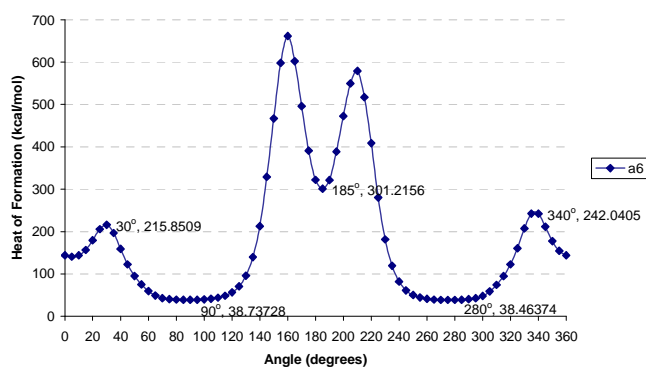


Chart 6. Rotational energy barriers for dihedral a6

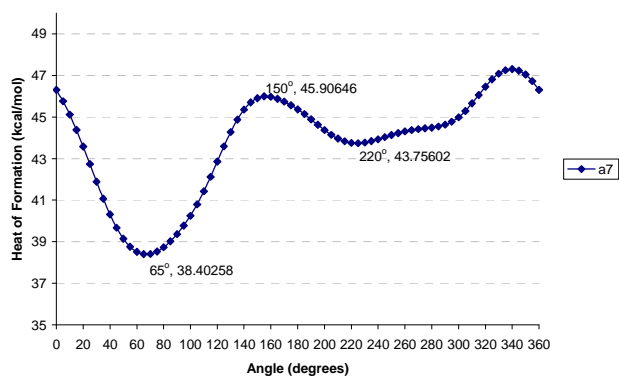


Chart 7. Rotational energy barriers for dihedral a7

4. Conclusions

In the present work, conformational analysis of all flexible bonds within the title compounds has been performed employing different methods: AMBER96 force field and semi-empirical AM1 and PM3 methods, implemented in two different molecular modeling software packages, HyperChem 7.5 and Mopac2009, respectively.

The comparison of the energetic barriers computed for AM1-based approaches has shown that the results are roughly similar, regardless of the molecular modeling software employed to perform the calculation. Additionally, similar geometries for the lowest energy conformations have been achieved for all three AM1-based approaches. In all these geometries an intramolecular H-bond has been detected between the carbonyl moiety of the

aromatic fused ring and the hydrazine hydrogen atom. Considerable differences have been noticed for PM3-based approaches, (3), (5) and (7), both in terms of conformers' geometry and calculated heat of formation, between the conformers generated via HyperChem 7.5 and subsequently, optimized by PM3 method implemented in Mopac2009 and the other two sets of conformers. In this case, a clear explanation for this discrepancy could not be found and needs to be further investigated. Nevertheless, for all three PM3-based methods the pyramidalization of nitrogen N10 has been noticed. This leads to an out of plane orientation of the H linked to this nitrogen atom and the intramolecular H-bond between the H atom and the carbonylic oxygen atom, O18, can not be formed. Probably, the absence of this intramolecular H-bond induces a destabilization to the system and at least for approaches (3) and (5) could explain the higher magnitudes observed for the heat of formation.

The lowest energy conformation obtained from approach (6) has been selected to compute rotational energy barriers against all seven rotatable bonds to discover the proper values of the corresponding dihedrals that could give information about the bioactive conformation and lead to the reduction of steric effects within molecule to be used in further molecular modeling studies. The computed energetic barriers for dihedral angles a1-a7 show that in some cases, e.g. a1 and/or a2, the "deep" valley on the surface of PES are populated with numerous low-energy conformations and it is possible to surmount the energetic barriers to pass from a low energy conformation to another.

Conversely, for dihedrals a5 and a6 the energy of rotational barriers is very high (in the range of hundreds) suggesting that the bioactive conformation of the ligand can not undergo conformational reorganization with respect to these dihedrals, due to steric effects and the lost of the intramolecular H-bond.

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