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Characterization of the conformational ensemble from bioactive *N*-acylhydrazone derivatives

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ABSTRACT

The search for bioactive conformations from prototypes is mostly referenced on crystallographic ligand–receptor complexes, in which the molecule conformation is already caged inside its binding site. However, the complexation process is a thermodynamic event depending on both complexed and uncomplexed states. As ligand affinity originates from such equilibrium, the development of novel computational models capable of supplying data on ligand dynamics in biological solutions is potentially applicable in more efficient methods for prediction of compounds binding and affinity. In this context, the current work employs a series of molecular dynamics simulations on three *N*-acylhydrazone derivatives, already shown to present promising cardioinotropic and vasodilatory activities, in order to obtain a precise characterization of each compound conformational ensemble in aqueous solutions, instead of a single minimum energy conformation. Consequently, we were able to observe the influence of each functional group of the studied molecules on the conformation of the entire compounds and thus on the exposure of functional groups that might potentially bind to target receptors. Additionally, the differences between the molecules conformational behavior were characterized, supporting a spatial and temporal image of each ligand, which may be potentially correlated to their biological activities. So in the context of conformational selection, such strategy may represent a useful methodology to contribute in the choice of ligands conformations for both 3D-QSAR and docking calculations.

1. Introduction

An important strategy in medicinal chemistry efforts to develop new bioactive compounds is the identification of the pharmacophore, a structural framework that comprises stereoelectronic properties and three-dimensional characteristics necessary to the complexation of ligands to a specific target receptor [1] and, consequently, to initiate a set of activities. An example of such group may be found in the *N*-acylhydrazone (NAH) moiety, already shown to be related to a series of biological activities such as analgesic, anti-inflammatory, and platelet aggregation inhibition activities [2–10], as well as protozoa proteases inhibition [11], HIV-1 reverse transcriptase dimmer destabilization [12], antibiotic and antifungal activities [13], and cardiovascular actions [14–18]. The prototype of this class of compounds, LASSBio-294, was first synthesized from natural safrole and identified as a potential alternative therapeutic for congestive heart failure due to a positive cardioinotropic effect through an interaction with the Ca^{2+} uptake/release process of the sarcoplasmic reticulum [14,15,17]. More recently, a vasodilatory action was also observed [16].

These diverse biological activities appear to be related to the same pharmacophoric group (in each case, added by specific substituents), rendering to the NAH moiety the status of privileged structure [7]. However, its conformational characterization as a function of biological solutions and specific or non-specific target receptors is highly difficult to be obtained under experimental techniques, as NMR and X-ray crystallography, due to few inter-proton distances and potential crystal packing effects [19,20].

One strategy usually employed attempting to circumvent such limitations in the conformational description of bioactive compounds consists in performing a conformational analysis. While

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such method, in its myriad of possible strategies, is usually concerned in medicinal chemistry to search for minimum energy structures, simulation techniques as molecular dynamics (MD) generate an ensemble of conformational states that include structures not at its energy minima [21]. The reliability of models built using computer simulation techniques depends mostly on (1) the size of the configurational space simulated, and (2) the accuracy of the force field employed to model the molecular system [22]. As a consequence, a tradeoff may be observed between accuracy of the force field on the one hand and the time scale that can be attained on the other. When properly validated, the additional kinetic energy due to temperature enhances the ability of the system to explore its potential energy surface and thus prevent the molecule to stay trapped in a localized region of the conformational space [21]. In this process, the explicit inclusion of solvent effects, as well as the evaluation of compounds conformation as an ensemble, supports their conformational description closer to a biological solution environment.

In this context, the current work intends to obtain a conformational characterization of the cardio- and vasoactive 2-thienylidene *N*-acylhydrazone derivatives LASSBio-294 (**1**) and LASSBio-785 (**3**) in explicit solvent, comparing their behavior with that displayed by furylidene isoster LASSBio-129 (**2**) [23]. Globally, the obtained results indicate that the conformational analysis of compounds, integrating quantum mechanical and simulation methods to consider both spatial and temporal components, may represent a promising tool for describing compounds conformational ensemble in biological solutions. Ultimately, it may contribute in building more accurate pharmacophoric models and, consequently, in an increased understanding of structure–activity relationships.

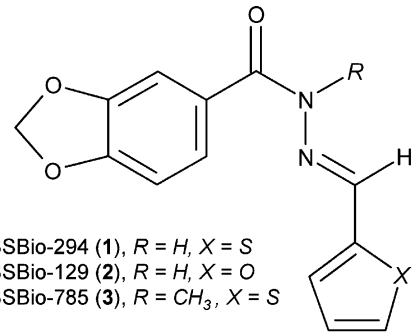
2. Experimental

2.1. Software

The compounds topologies were generated with the PRODRG server [24], the *ab initio* calculations were performed using GAMESS [25], the semi-empirical calculations were performed using MOPAC2009 [26], manipulation of structures was performed with MOLDEN [27], and all the MD calculations and analysis were performed using the GROMACS simulation suite [28] and GROMOS96 force field [29], as an inexpensive and fast simulation package.

2.2. Conformational analysis strategy

In order to obtain a detailed picture of NAH conformational features, as well as its dependence on bounded substituents, the molecules (Fig. 1) were divided as building blocks (named blocks I–III) [30]. Such strategy intends to support both the conformational characterization of the NAH derivatives, with a progressive level of complexity, and the isolation of specific conformational attributes added by each of the compounds functional groups. In this context, block I is composed by the NAH unit or by the *N*-methyl-*N*-acylhydrazone unit (NMNAH), whose degrees of freedom were named ϕ_1 and ϕ_2 (Fig. 2 A). The inclusion of the 1,3-benzodioxolyl ring on such moieties added the ϕ_3 dihedral and defined block II (Fig. 2B). Finally, the addition of 2-thienyl or 2-furyl rings created the ϕ_4 dihedral, and this complete form of the compounds was named block III (Fig. 3C). Additionally, as the synthesis of NAH derivatives is described to produce mainly the (*E*)-diastereoisomers [4,18,23], only the isomer presenting this relative configuration was considered for the studied compounds. Based on this fragmentation strategy, each of the above-mentioned degrees of freedom was sampled by semi-empirical calculations to accordingly include quantum mechanical parameters. In sequence, each of these minimum energy conformations was submitted to microsecond MD simulations in explicit solvent in order to describe the conformational behavior of each molecule in aqueous solutions. The details of these proceedings are described below in the text.



LASSBio-294 (**1**), *R* = *H*, *X* = *S*
LASSBio-129 (**2**), *R* = *H*, *X* = *O*
LASSBio-785 (**3**), *R* = CH_3, *X* = *S*

	1	2	3
Vasodilatory (IC_{50})	74 μM	*n.d.*	10.2 ± 0.5 μM
Analgesic (ID_{50})	8.1 μmol.kg^{-1}	*n.d.*	*n.d.*
Anti-platelet (IC_{50})	15.3 μM	*n.d.*	*n.d.*
Anti-inflamatory (% of inhibition)	29.7	*n.d.*	*n.d.*

n.d. = not determined;

Fig. 1. The structure of the studied molecules: LASSBio-294 (**1**), LASSBio-129 (**2**) and LASSBio-785 (**3**), together with their biological activities, as determined [7,10,16,18].

2.3. Search for minimum energy conformations on each block

The structures in each of the three above-mentioned blocks (Fig. 2) were submitted to full-geometry optimization using RM1 Hamiltonian semi-empirical method [31] at the SCF-MO level in the gas phase with MOPAC2009. Hessian matrix analyses were employed to unequivocally characterize the obtained geometries as true minima potential energy surface [32]. Each degree of freedom, as identified in Fig. 2, was rotated from 0 to 360° with a 30° step. The obtained conformers were energy optimized using restraints only for the studied dihedrals, allowing the rest of the molecule to be minimized. The use of RM1 semi-empirical calculations, as well as other newer Hamiltonians, PM6, support a fast geometry optimization and account for high resolution structures, frequently in best agreement to experimental data then high level Hartree–Fock calculations [33–35]. Additionally, systems composed by compounds **1**–**3** in the presence of explicit water molecules, obtained from MD simulations, were submitted to geometry optimization under MOZYME procedure. For comparison, all semi-empirical calculations in the absence of explicit solvent molecules were also performed under PM6 method [36] and under COSMO continuum model [37] (supplementary data).

2.4. MD simulations

The minimum energy conformations obtained from RM1 semi-empirical calculations were used as starting points for a series of MD simulations. Such structures were submitted to the PRODRG site, and the initial geometries and crude topologies under the GROMOS96 force field were retrieved. These topologies were modified to include atomic charges following a proceeding previously described by our group [38–40]. Such protocol was shown to be able to offer an adequate conformational representation of highly charged compounds as carbohydrates and glycosaminoglycans, as well as its binding to target receptors, with a lower