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Original article

Physicochemical characterization of neuroleptics. Relative stability of 7- and 9-hydroxyrisperidones and their protonated forms in gas phase and in solution

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ABSTRACT

Conventional *ab initio* and DFT-B3LYP calculations have been used to investigate on molecular conformation, dipole moments, intramolecular hydrogen bond and relative energies of 9-hydroxy and 7-hydroxyrisperidone metabolites of risperidone, in their neutral and mono-protonated forms in the gas phase and in solution. Three minimum energy conformations characterize the potential energy surface of 7-hydroxyrisperidone, while 9-hydroxyrisperidone is dominated by a strong intramolecular OH···N hydrogen bond of ca. 8 kcal/mol, which drastically reduces in water solution. In the gas phase, 9-hydroxyrisperidone is the most stable isomer both in the neutral and in the protonated forms. Solvent effects favour 7-hydroxyrisperidone rotamers owing to their higher dipole moment values. Under the physiological pH of 7.4, the protonated forms of both isomers in water coexist in almost equivalent amount, in qualitative agreement with the experiment. It is suggested that to stabilize the pharmacologically active 9-hydroxyrisperidone over the 7-OH isomer one has to increase its molecular dipole moment and the intramolecular OH···N hydrogen bond energy.

1. Introduction

Risperidone (RSP), 3-[2-[4-(6-fluoro-1,2-benzisoxazole-3yl) piperidin-1-yl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one, is an effective second generation antipsychotic drug which is used world-wide for the treatment of psychotic diseases. Risperidone is metabolized in the lever by cytochrome P450 via alicyclic hydroxylation of the tetrahydropyrido[1,2-a]pyrimidin-4-one (TPP) moiety and N-dealkylation of the piperidine nitrogen [1]. Hydroxylation produces mainly 9-OH-RSP together with a small amount of 7-hydroxy isomer, Fig. 1. Both compounds are present in the (+) and (−) enantiomeric forms which can be separated by enantioselective techniques of analysis [2,3]. The pharmacological activity of 9-OH-RSP is considered to be similar to that of the parent compound [4]. Following Mannens et al. [1], in urine 9-OH-RSP accounts for 31% of the administered dose, while 7-OH-RSP accounts for ca 5% of the dose. In the plasma the corresponding values are 54% and 3%, therefore the 7-OH-RSP to 9-OH-RSP concentration ratio varies in a significant range with a non-negligible percentage of the 7-OH isomer. The metabolism of 9-OH-RSP in humans was recently studied [5]. It is metabolized to a limited extent. No metabolites could be detected in plasma. In urine four metabolites were detected representing relatively small amount of the administered dose (3–5%). Biotransformation occurs via oxidative-N-dealkylation, monohydroxylation of the alicyclic ring, alcohol dehydrogenation and benzoisoxazole scission [5].

The pharmacological properties of 7-OH-RSP have not been investigated, thus, to avoid overestimation of 9-OH-RSP concentration, the two hydroxyl isomers need to be separated prior to their determination, as they cannot be distinguished in the usually employed mass spectrometry measurements [6]; the separation is not a straightforward matter and much effort has been made to determine risperidone and its degradation products in various substrates employing a variety of experimental techniques [7–13]. Knowledge of the physicochemical properties of the OH-RSP isomers may help choice and use of analytical methodologies and would probably assist further advance in pharmacokinetics understanding of both isomers. Indeed, detailed thermodynamic data for the formation of hydroxyl metabolites of risperidone are lacking. The present work investigates on the relative stability of 7-OH-RSP and 9-OH-RSP and their protonated forms in the gas phase and in water solution, aiming to elucidate factors which determine it. We used a computational approach based on conventional *ab initio* and Density Functional Theory (DFT) methods.

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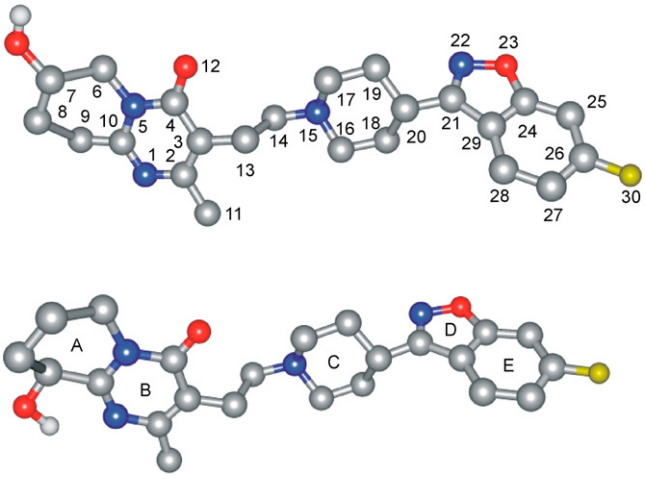

Fig. 1. Molecular structures of 7-hydroxyrisperidone and 9-hydroxyrisperidone.

2. Computational details

All calculations were performed with the Gaussian 03/E.01 package [14]. Molecular structures were optimized by DFT procedures using the B3LYP functional [15] with the 6-31G* basis set. The relative free energy ΔG of a pair of systems was obtained as:

$$\Delta G = \Delta E + \Delta ZPVE + \Delta H_{vib}(T) - T\Delta S(T) \quad (1)$$

where E is the internal energy, ZPVE is the non-scaled relative zero point vibrational energy in the double harmonic approximation and $H_{vib}(T)$ and $S(T)$ are the vibrational enthalpy and the total entropy at $T = 298$ K, respectively. $\Delta H_{vib}(T)$–$T\Delta S(T)$ stands for the so-called thermal correction term.

Bulk solvent effects were accounted for by the self-consistent reaction field approach based on the Polarised Continuum Model (PCM) method [16], which has been proven to be an effective tool to investigate on a variety of solution phase physicochemical properties [16,17]. In the model, the United Atom Topological Model applied on cavity radii optimized for the HF/6-31G* level of theory was used as implemented in the Gaussian program.

3. Results and discussion

3.1. Conformational behaviour of 7-hydroxy and 9-hydroxytetrahydropyrido[1,2-a]pyrimidin-4-ones (7-OH-TPP and 9-OH-TPP) as model compounds

Ab initio calculations on molecular geometries and relative stabilities of 9-OH-RSP and 7-OH-RSP were carried out at the B3LYP/6-31G* level of theory, which is considered adequate to study conformational behaviour in medium-sized molecular systems [18]. To save computational time, needed extensive calculations on the OH group conformational behaviour and some higher level correlated calculations were carried out on the OH-TPP molecular moiety. The absence of conjugative interactions between OH-TPP and the remaining molecular framework, led us to consider that structural and conformational features exhibited by OH-TPP are essentially as in OH-RSP.

Minimum energy conformations and height barriers associated with the OH rotation were obtained from full relaxed potential energy surface (PES), both in the gas phase and in water solution. The results are shown in Figs. 2 and 3. Relative energies, obtained in steps of 30° of torsional angle ϕ in the 0–360° range, are very well fitted by a truncated Fourier-like expansion [19].

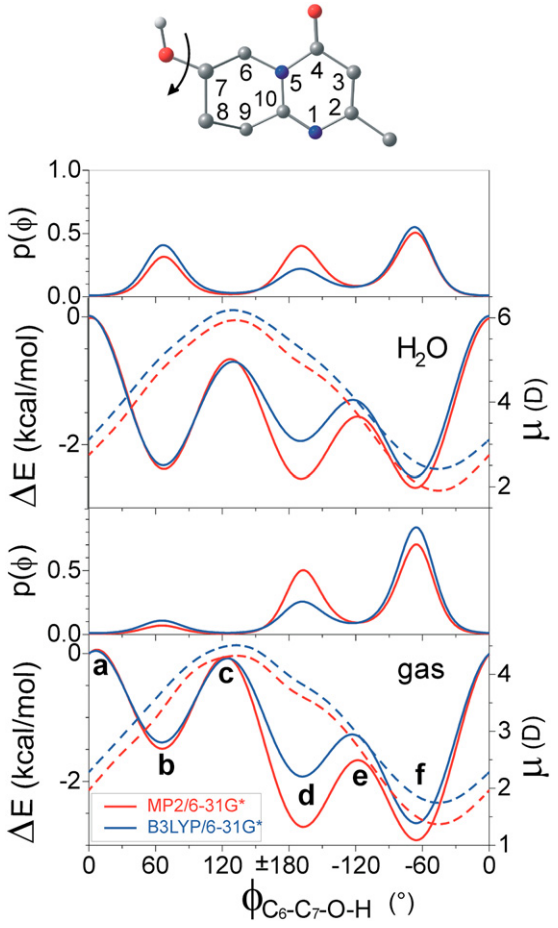

Fig. 2. Torsional potentials, rotational probabilities and dipole moments (dashed lines) of 7-OH-TPP as a function of the $\phi_{C6-C7-O-H}$ dihedral angle.

$$V(\phi) = V_0 + \sum_{n=1}^{4} V_n \cos(n\phi) + \sum_{n=1}^{4} V_n' \sin(n\phi) \quad (2)$$

The rotational probability $p(\phi)$ to find the molecule at a torsional angle ϕ, at the body temperature of 37 °C, was obtained according to a Boltzmann distribution of conformational energies:

$$p(\phi) = \frac{e^{-V(\phi)/RT}}{\int_0^{2\pi} e^{-V(\phi)/RT} d\phi} \quad (3)$$

The results that characterize critical points in PES are summarized in Tables 1 and 2. The minimum energy conformation of 7-OH-TPP in vacuum is obtained at $\phi_{C6-C7-O-H} = -66°$ (conformer **f**). Two further stable conformations are found at $\phi_{C6-C7-O-H} = -167°$ (conformer **d**) and 66° (conformer **b**). Populations of these minimum energy conformations, as obtained by ΔE values assuming a Boltzmann distribution of the conformers

$$N_i = \frac{e^{-\Delta E_i/RT}}{\sum_i e^{-\Delta E_i/RT}} \quad (4)$$

are 5.4%, 39.3% and 55.3% at MP2/6-31G* level and 8.9%, 21.3% and 69.8% at B3LYP/6-31G* level. These figures compare well with those obtained by integration procedure from the peak