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COMMUNICATION

Is nevirapine atropisomeric? Experimental and computational evidence for rapid conformational inversion†

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The non-nucleoside reverse transcriptase inhibitor nevirapine displays in its room temperature ^{1}H-NMR spectrum signals characteristic of a chiral compound. Following suggestions in the recent literature that nevirapine may display atropisomerism—and therefore be a chiral compound, due to slow interconversion between two enantiomeric conformers—we report the results of an NMR and computational study which reveal that while nevirapine does indeed possess two stable enantiomeric conformations, they interconvert with a barrier of about 76 kJ mol^{-1} at room temperature. Nevirapine has a half life for enantiomerisation at room temperature of the order of seconds, is not atropisomeric, and cannot exist as separable enantiomers.

Introduction

Nevirapine (Fig. 1) is a non-nucleoside reverse transcriptase inhibitor developed by Boehringer Ingelheim in the 1990's.[1] It received approval for use in the treatment of HIV between 1996 and 1998 and its efficacy has been explored in a number of clinical trials since then, some of them controversial.[2] Nevirapine's X-ray crystal structure[3] reveals a butterfly-like shape in which the planar pyridyl rings are bent upwards or downwards from the puckered central diazepinone, their planes intersecting at an angle of 121°. Despite potential delocalisation of its lone pair into the adjacent pyridyl rings, the nitrogen atom of the cyclopropylamine is significantly pyramidalised, and the cyclopropyl ring adopts a position almost perpendicular to the plane of the diazepinone, opposite to the two pyridyl rings. Vibrational spectroscopy and computational studies confirm a similar conformation in solution and when bound to the drug's protein target.[4]

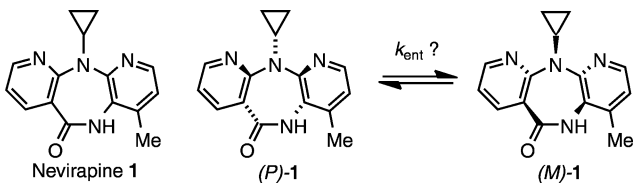

Fig. 1 Nevirapine and its enantiomeric conformers.

Despite nevirapine's lack of stereogenic centres, this favoured conformation has no plane of symmetry, and can interconvert with its mirror image by flexing of the diazepinone, with inversion of the nitrogen atom accompanied by a butterfly-wing motion of the pyridyl rings as the cyclopropyl group passes between the pyridyl nitrogen atoms. The relatively slow interconversion of the enantiomeric conformers of benzodiazepinones has been exploited for the enantioselective construction of quaternary stereogenic centres by "memory of chirality."[6] Furthermore, related benzolactams (albeit with a fully substituted lactam N) display atropisomerism[5]—that is, the interconversion of their conformers is slow enough for those conformers to exist as separable, chiral stereoisomers. Structurally related sterically encumbered aromatic amides,[7] anilides,[8] ureas,[9] and (thio)ethers[10] may also display atropisomerism.

As with all chiral compounds, atropisomers may display biological activity that is dependent on their absolute configuration[11] and for this reason unforeseen atropisomerism has recently been highlighted as a potential pitfall in the development of drug candidates.[12] Although conformational motion in the cyclopropyl sidechain of nevirapine has been studied computationally[4] it is remarkable that the rate of conformational inversion of nevirapine k_{ent}, and whether it may be low enough for enantiomeric atropisomers to exist, has never been reported.

The question of atropisomerism in nevirapine was further highlighted early in 2011 in a paper,[13] since withdrawn,[14] claiming an optical rotation for a sample of nevirapine purportedly isolated from a natural source—something possible only if nevirapine can indeed exist as a pair of atropisomeric enantiomers. In response to this paper, and to the discussion it generated,[15] we have quantified, by spectroscopic and computational methods, the barrier to conformational inversion of nevirapine, and in this paper we report our results.

Results and discussion

NMR spectroscopy

Lack of planarity in the diazepinone ring is evident in the ^{1}H NMR spectrum of nevirapine. Although the initial characterisation of the molecule reported multiplets (3.62, 1H, H_X; 0.88, 2H, $H_{A,B}$; 0.35, 2H, $H_{C,D}$) for the protons around the cyclopropyl ring, close

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Table 1 Chemical shifts and coupling constants at 303 K for the protons around the cyclopropyl ring

	δ (ppm)	J_A/Hz	J_B/Hz	J_C/Hz	J_D/Hz
H_A	0.2925	—	10.5	–4.8	6.5
H_B	0.3665	10.5	—	6.5	–4.8
H_C	0.8686	–4.8	6.5	—	9.0
H_D	0.8961	6.5	–4.8	9.0	—
H_X	3.6151	3.9	3.9	6.7	6.7

inspection of the ^{1}H NMR spectrum of a sample of nevirapine (0.02 M in d_6-DMSO) at room temperature revealed symmetrical bandshapes at 0.88 and 0.35 ppm corresponding to the ABCD region of an ABCDX system in which coupling constants are symmetry-related but chemical shifts are not. The chemical shifts and coupling constants shown in Table 1 were determined by comparison with simulation. The pairs of protons $H_{A/B}$ and $H_{C/D}$ are distinguished by lying *trans* or *cis* to the cyclopropylamine nitrogen whatever the symmetry of the rest of the molecule, but the chemical inequivalence of H_A and H_C, and H_B and H_D, excludes a plane of symmetry through the rest of the molecule, rendering these pairs diastereotopic.

Inversion of the nitrogen and flipping of the diazepinone (see Fig. 2) leads to exchange of the protons H_A and H_B, and H_C and H_D, resulting in an AA′BB′X spin system under fast exchange, and we set out to determine the temperature dependence of the rate of exchange by dynamic NMR techniques. ^{1}H NMR spectra were acquired in d_6-DMSO at a range of temperatures from 298 K (25 °C) to 390 K (117 °C). Coalescences were observed between H_A and H_B, and H_C and H_D at around 60–70 °C, indicating a shift from the slow to the fast exchange regime.

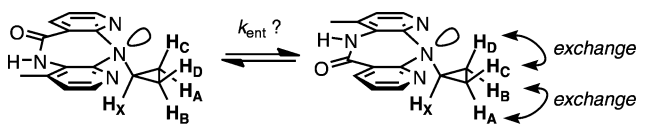

Fig. 2 Chemical exchange and conformational inversion of nevirapine and its enantiomeric conformers.

The bandshapes over the full temperature range were modelled both by direct density matrix calculation using Mathematica[16] and by using the commercial program gNMR.[17] The linewidth was set at 1 Hz, and the average chemical shifts for protons A and B and protons C and D were changed with temperature to match the positions of the two experimental bandshapes. The rate of exchange was adjusted by eye to give the best fit between the experimental and modelled lineshapes for both multiplets at each temperature (Fig. 3).

The value of k at 60 °C, where the line shape is most sensitive to variations in k, is 19 ± 4 s^{-1}, which corresponds to a value of $\Delta G^{\ddagger}$ for the enantiomerisation process of 73.7 ± 0.45 kJ mol^{-1}. By plotting $\ln(k/T)$ against $1/T$ for data points close to coalescence we estimated values for $\Delta H^{\ddagger}$ of 81 ± 1.4 kJ mol^{-1} and $\Delta S^{\ddagger}$ of +22 ± 5.4 J mol^{-1} K^{-1}. While we have high confidence in the rate of exchange, and therefore the value of $\Delta G^{\ddagger}$, close to the coalescence point, the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ estimated by this method are considerably less certain, because of the unusual nature of the underlying spectrum. The large number of individual transitions contributing to this very strongly coupled spectrum

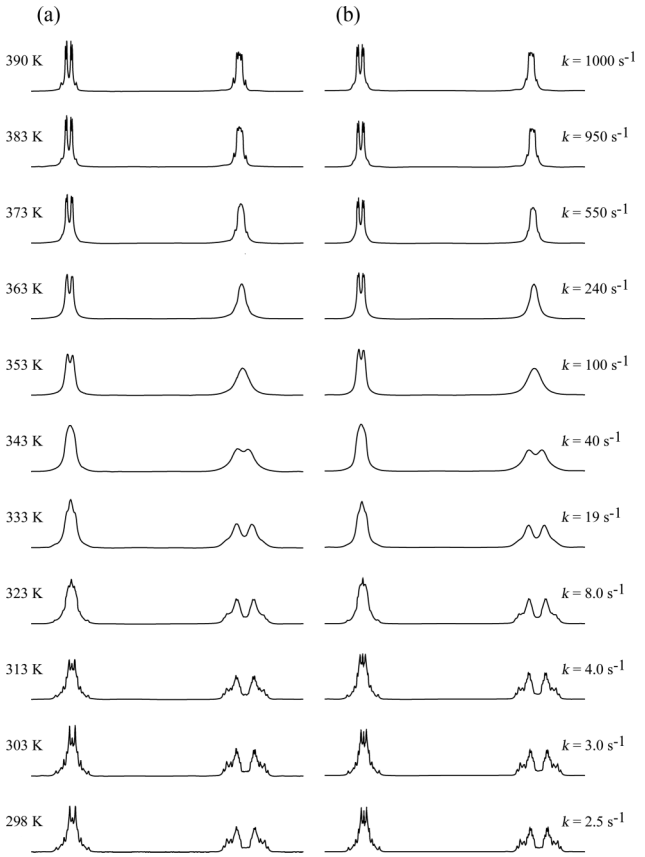

Fig. 3 (a) Experimental and (b) modelled bandshapes for H_A-H_X.

means that the bandshape at low and high temperatures is very sensitive to instrumental line broadening and T_2 relaxation, while above coalescence there is no direct means of assessing any changes in the chemical shift differences between protons A and B and C and D.

Nonetheless, a value of $\Delta S^{\ddagger}$ of this magnitude suggests that $\Delta G^{\ddagger}$ for the enantiomerisation of nevirapine varies between 71 and 75 kJ mol^{-1} over the range of temperatures studied. At 25 °C, we estimate a half-life for the enantiomerisation process of 1.5 s, or a half life for racemisation ($k_{rac} = 2k_{ent}$) of less than a second. Nevirapine is not atropisomeric at room temperature, and would be separable into enantiomeric atropisomers only below about –30 °C.

Computation

To illuminate the process by which conformational inversion occurs, and to model the barrier computationally, electronic structure calculations, using the Gaussian09 suite of programs,[18] were carried out using density functional theory methods to map out the potential energy surface (PES) across which inversion at the nitrogen atom occurs. We first explored the main features of the PES using the M06-L functional in conjunction with a 6-31G** basis. Thermodynamic corrections were evaluated using the rigid rotor, harmonic oscillator approximation to give the free energies that we quote here.

The initial step from the minimum energy structure (Fig. 4a) leads to the transition structure (TS) (Fig. 4b), which is close to planarity at the inverting nitrogen. The corresponding barrier

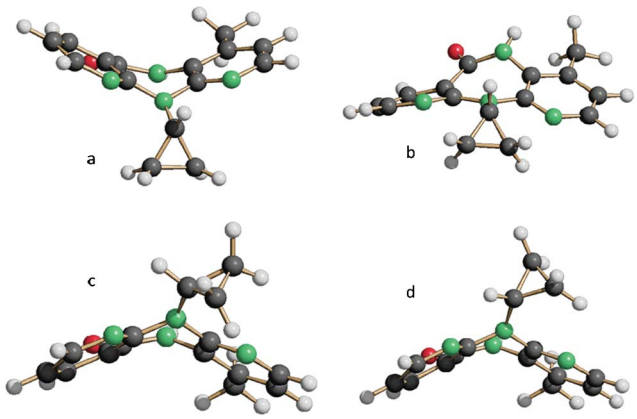

Fig. 4 The global minimum (a), the inversion transition state (b), the local minimum (c) and the transition state from the local minimum to the global minimum (d).

is calculated to be 58 kJ mol^{-1}. This TS leads to a local energy minimum (Fig. 4c) 18 kJ mol^{-1} above the global minimum. Rotation of the cyclopropyl group is needed for the global minimum energy structure to be reached again. The barrier for this step was calculated to be 16 kJ mol^{-1}, this TS (Fig. 4d) being 22 kJ mol^{-1} below the TS for nitrogen inversion. We note that there is an alternative local energy minimum to 4c, in which the cyclopropyl group is rotated, and whose energetics are close to those of 4c.

Since the initial barrier to inversion at the nitrogen will determine the kinetics observed experimentally, we have evaluated this barrier using a more realistic model. We have employed a 6-311G(2D, 2P) basis together with the M06-2X functional, which is more computationally demanding than M06-L, but has been shown to describe long range interactions more accurately.[19] Solvation effects were included firstly by adding a single DMSO molecule, whose general position, hydrogen-bonded to the N–H group, was previously identified from molecular dynamics simulations.[20] The remaining bulk solvation was included by a single point calculation using the CPCM model employing a dielectric of 46.8 (DMSO). This procedure led to a free energy barrier to nitrogen inversion of 76 kJ mol^{-1}.

Conclusion

Nevirapine exists as two enantiomeric non-planar conformers which interconvert with a barrier of about 75 kJ mol^{-1} at room temperature, determined both experimentally and computationally. We therefore conclude that while nevirapine's room temperature NMR spectrum displays asymmetry, it is not an atropisomeric compound at this temperature and cannot be isolated as a single enantiomer above a temperature of about –30 °C.

Acknowledgements

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