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In search of the mutual relationship between the structure, solid-state spectroscopy and molecular dynamics in selected calcium channel blockers

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ABSTRACT

Three isostructural 1,4-dihydropyridines (DHPs), namely, nifedipine, nitrendipine and nimodipine were selected to characterize their structure, intermolecular interactions and molecular dynamics. The studied samples were analyzed using powder X-ray diffraction (XRD), neutron (INS) and infrared spectroscopy (FT-IR) as well as solid-state nuclear magnetic resonance (NMR), where each technique was supported by the state-of-the-art theoretical calculations for solid-state. By combining multiple experimental techniques with advanced theoretical calculations we were able to shed light on the mutual relation between the structure, stabilizing intermolecular interactions and their spectral response. For the first time, unambiguous computationally-supported assignment of the most prominent spectral features in DHPs is presented to give a valuable support for polymorph screening and drug control. Molecular motions were interpreted in details, revealing that a dynamic reservoir of each compound is dominated by intra-molecular reorientations of methyl groups and large-amplitude oscillations in terminal chains. Our study successfully validates the realm of applicability of first-principles solid-state calculations in search of the mutual relation between the structure and spectroscopy in this important class of drugs. Such approach gives a first necessary step to gather combined structure-dynamics data on functionalized DHPs, which are of importance to better understand crystallization and binding tendency. The NMR relaxation experiments reveal that nitro groups significantly hinder the reorientation of methyl rotors and provide the first evidence of low-temperature methyl-group tunneling in DHPs, an intriguing quantum-effect which is to be further explored.

1. Introduction

It is well known that most drugs are formulated and ingested as solids (Allen, 2008). The ability to effectively deliver solid pharmaceuticals strongly depends upon the form of drug in solid state. In that regard, knowledge of actual arrangement adopted by drug molecule in crystal lattice is important to establish the mutual structure–property relationship as well as to control its bioavailability (Xu et al., 2012; Veber et al., 2002). Among multiple physical chemical techniques, optical vibrational spectroscopy is the method of choice in research of active pharmaceutical ingredients (API), with a great potential of use in purity and polymorph screening or monitoring of the interactions between a drug molecule and a carrier (Caliandro et al., 2013; Wang et al., 2011). Vibrational response of an API expresses direct link between intermolecular interactions and structural arrangement, which is of great importance owing to the unique properties that each pharmaceutical polymorph possesses. In addition to this static view, crystallization or amorphization tendency as well as receptor affinity is defined by dynamic reservoir of its molecular framework, which competes with intermolecular interactions. Temperature-driven molecular dynamics makes an understanding of molecular pharmaceutics extremely challenging. Despite of its complexity, the problem of mutual structure-dynamics correlation is of great importance to pharmaceutical science as giving perspective of more conscious and more effective design of novel drugs (Paudel et al., 2014).

1,4-dihydropyridine derivatives constitute a very important class of drugs that are present on the pharmaceutical market in solid form. They work by blocking the flow of calcium ions into the cell, which reduces the contractility of the heart and smooth muscle tone in the coronary arteries and results in a reduction in myocardial oxygen demand (Yamamura et al., 2013; Lovakovic et al., 2011). They exert potent effects on blood vessels, therefore, tend to be mainly used in treatment

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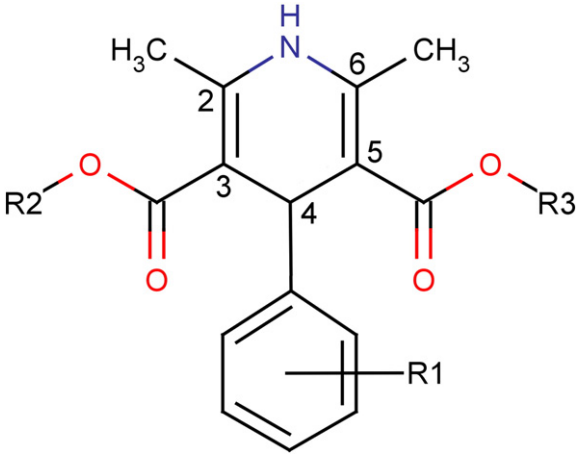
of hypertension and coronary heart disease (Smolensky et al., 2010). Among numerous disease entities calling for the use of DHPs, one could mention systolic hypertension or Prinzmetal's angina (Frishman and Michaelson, 1997). In addition, there are multiple pleiotropic beneficial effects of their use, including effects on the vascular endothelium and release of nitric oxide, inhibition of platelet aggregation or antiatherosclerotic and antioxidant activity (Yamagishi et al., 2006; Berkels et al., 2003; Vijesh et al., 2008). There are also reports on the beneficial effects of DHP derivative in treatment of arrhythmias associated with myocardial ischemia or in atherosclerosis (Ishii et al., 2012; Napoli et al., 2005).

The analysis of the structure–activity relationship in DHPs has been presented in Ref. Triggle, (2003) and Pedemonte et al., (2007) as delivering several crucial conclusions. It was found that: (I) the presence of 1,4-dihydropyridine ring is essential and the substitution of phenyl ring at C4 position leads to optimum pharmacological activity. (II) Further substitution of the phenyl ring (R1) with electron-donating group is very important, where *orto*- and *meta*-embedding was found to be more effective, due to electronic-structure and steric issues. Most DHPs consist of nitro substituents, which, however result in low photo-resistance. (III) It was found that the 2,6–substituents in 1,4-DHP ring should consist of lower alkyl groups, while ester groups at the C3 and C5 position show optimal activity (see R2 and R3). (IV) It was also found that ester substitution larger than $COOCH_3$, maintain, or even increases the activity because of bulk tolerance in the site of 1,4-DHP. For that reason, the 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate core (see Scheme 1) is a common feature of most of the DHPs used in therapy (Reimao et al., 2010).

Solid-state environment can be treated as a well-defined reference model, which allows one to understand more clearly the competing intermolecular forces in pharmaceutical solids. It is therefore interesting to disclose factors driving the crystal packing at the molecular level as well as to expose the spectral signatures and the molecular dynamics response of DHPs.

To this end, we have selected three functionalized DHPs, with similar chemical structure (see Scheme 2), namely nifedipine (NIF), nitrendipine (NTR) and nimodipine (NIM), which are commercially available calcium channel blockers used for the treatment of cardiovascular diseases (Inzitari and Poggesi, 2005; St-Onge et al., 2014). Each compound shares the same molecular core, differing in the nitro-phenyl substitution (*orto* — NIF; *meta* — NTR, NIM) as well as in the chemistry of the ester groups (C3 and C5) (Mitrega et al., 2012). As has been shown by Morales-Rıos et al. (2007), the chemical similarity of DHPs results in similar molecular conformation and crystal packing, mostly, in the preferable monoclinic $P2_1/c$ space group. For that reason, we have selected polymorphs crystallized in that form.

This work seeks to provide a sound description of, both, static–structural and dynamic properties of selected calcium channel blockers, supported by modern solid-state oriented quantum chemical calculations. In that way, we are able to provide a reliable analysis of the structural features along with concomitant spectral signatures, which may be helpful *e.g.* in polymorph discrimination and drug processing. To this end, we extend the analysis onto the intermolecular interactions driving the studied crystal structures, which are then characterized by computationally-supported combination of infrared (IR) and high-resolution solid-state nuclear magnetic resonance (NMR) spectroscopy. Finally, with the help of relaxation NMR experiments we have followed their molecular dynamics across a broad temperature range. The intramolecular motions were eventually assigned with the help of theoretical calculations. Such a task is facilitated by the absence of structural disorder and low-temperature phase transitions and represents the first necessary step to gather the combined structure-dynamics data on functionalized DHPs.



Scheme 1. The most common framework (4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) of DHP derivatives used in medical treatment.

2. Experimental

2.1. Materials

The high-purity samples were kindly provided by Alfa Aesar GmbH & Co., Germany (nifedipine), Toronto Research Chemicals Inc., Canada (nitrendipine) and Sigma Aldrich, USA (nimodipine).

Powder X-ray diffraction (XRD) analysis of each compound was carried out on an Empyrean (PANalytical) diffractometer, using a CuKα radiation (1.54 Å), reflection-transmission spinner and PIXcel 3D detector, operating in the Bragg–Brentano geometry. The continuous 2Θ scans were collected at room temperature, in the range of 5–60°, with a step size of 0.013°.

The phase stability in each sample was controlled using different scanning calorimetry (DSC), with a DSC 8000 calorimeter (Perkin-Elmer) at the heating and cooling rate of 10.0 K min^{-1}, by scanning the samples from 173 K up to the melting point.

2.2. Solid-state spectroscopy

All the solid-state spectroscopy measurements were performed on the powder samples.

The middle-FT-IR spectroscopy was applied in a transmission mode, using a Bruker 66v/S FT-IR spectrometer, working with a deuterated triglycine sulfate (DTGS) detector and a silicon carbide (Globar) source. The spectrum was recorded using KBr pellets, by collecting 64 scans with a spectral resolution of 1 cm^{-1}, covering the wavenumber range of 4000–400 cm^{-1}.

In addition, the inelastic neutron scattering experiment for nimodipine was performed using inverted-geometry spectrometer NERA, (Natkaniec et al., 2014) set at the high flux pulsed nuclear reactor IBR-2 at JINR Dubna, Russian Federation. The incident neutron energies were determined by measuring the neutron time-of-flight across the 110 m distance from the IBR-2 water moderator to the sample. The INS spectra were recorded simultaneously for all the wavelengths/energies and averaged over fourteen scattering angles from 20° to 160°. The INS spectrum was collected at the final energy of the scattered neutrons $E_f = 4.65$ meV, fixed by crystal analyzers and beryllium filters. The ~5 g sample in a flat aluminum container was measured for 14 h at 295 K and 40 K, respectively. The neutron powder diffraction (NPD) patterns were recorded simultaneously, proving that no structural phase transition could be found below the room temperature. The recorded INS spectra were finally transformed into the form of generalized vibrational density of states $G(\omega)$ (Bokhenkov et al., 1976).

^{13}C CP/MAS NMR spectra were acquired using an Agilent spectrometer operating at Larmor frequency of 400 MHz for protons. The samples