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Preparation and Properties of New Co-crystals of Ibandronate

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Abstract: Polymorphism of active pharmaceutical ingredients (APIs) gets increasing attention as an important physico-chemical parameter influencing bioavailability and stability of API and pharmaceuticals. Co-crystals of API with common pharmaceutical excipients become very important as a tool to tune up solubility and absorption. Bisphosphonates (e.g. alendronate, risedronate, ibandronate) are widely used in clinical practice. They are indicated for the treatment and prevention of osteoporosis. They are powerful inhibitors of bone resorption, but their gastrointestinal adsorption is only about 1% due to their high hydrophilicity. Some experiments were designed to prepare co-crystals of ibandronate. In the present study various mixtures of ibandronate and excipients were prepared. All the prepared mixtures (solid compounds) and/or new entities were analyzed by means of FT-NIR spectroscopy. The absorption of potential new co-crystals was investigated by means of the PAMPA experiments.

Keywords: Bisphosphonates; Co-crystals; FT-NIR; PAMPA.

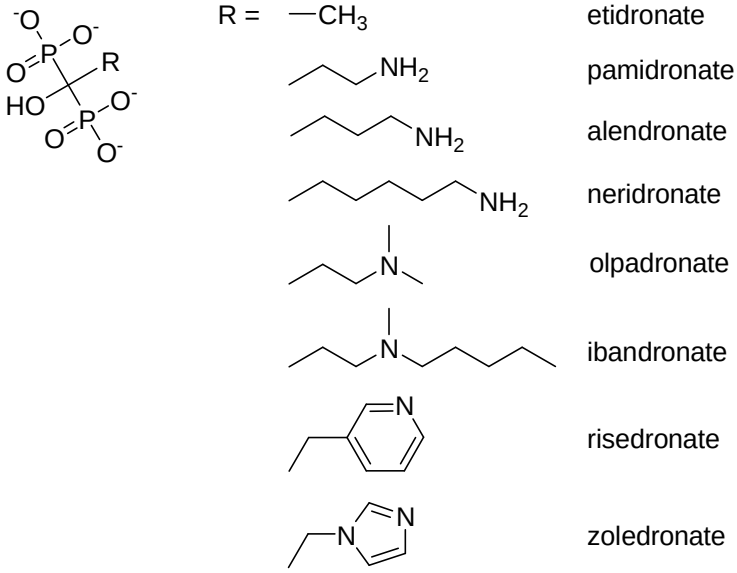
INTRODUCTION

Bisphosphonates (BPs) are the most widely used and the most effective antiresorptive agents currently available for the treatment of Paget's disease, tumor-associated bone disease, and osteoporosis. All BPs have high affinity for bone mineral as a consequence of their P-C-P backbone structure, which allows chelation of calcium ions [1]. The following release from bone mineral during acidification by osteoclasts, BPs appear to be internalized specifically by osteoclasts but not other bone cells [2]. The intracellular accumulation of BP leads to

inhibition of osteoclast function, due to changes in the cytoskeleton, loss of the ruffled border [2,3], and apoptosis [4-7]. The ability of BPs to inhibit bone resorption depends on the presence of two phosphonate groups in the P-C-P structure, which appear to be required for interaction with a molecular target in the osteoclast as well as for binding bone mineral [8-10].

Bisphosphonates as pyrophosphate analogues (see general structure in Figure 1) are a group of drugs widely used in practice. There are several injectable bisphosphonates: etidronate (Didronel®), pamidronate (Aredia®) and zoledronate (Zometa®), which may be given every three months or yearly. Ibandronate in contrast with alendronate (Fosamax®) and risedronate (Actonel®), which can be taken daily or weekly, is the only oral revolution biphosphonate that is approved to be taken monthly [11]. Oral bioavailability of these bisphosphonates is very low (their gastrointestinal absorption is about 1%) due to their high hydrophilicity [12].

Figure 1. Structures of clinically used bisphosphonates.



In general, structural modifications are the best way to improve permeability: *i*) ionisable groups to non-ionizable groups; *ii*) increase of lipophilicity; *iii*) isosteric replacement of polar groups; *iv*) esterification of carboxylic acid; *v*) reduction of hydrogen bonding and polarity; *vi*) reduction of size; *vii*) addition of a nonpolar side chain; *viii*) preparation of prodrugs. Generally these strategies are based on a few fundamental concepts: reduction of ionizability, increase of lipophilicity, reduction of polarity, or reduction of hydrogen bond donors or acceptors. Thus, it is important to assess permeability early and to build permeability improvement into the synthetic plan from the beginning. This could rescue a chemical series that has great potential and improve drug exposure in animal pharmacology and pharmacokinetic studies [13].

Formulation is other strategy for improving permeability and bioavailability. For example, permeability enhancers, surfactants or pharmaceutical complexing agents can be used in oral dosage form [13].

The application of co-crystal technologies has only recently been recognised as a way to enhance solubility, stability and the IP position with respect to the development of APIs. Unlike salt formation, co-crystallisation does not rely on ionisation of the API and the counterion to make a solid. Instead, both components utilise prominent intermolecular interactions, such as hydrogen bonding, to combine and yield a uniform crystalline material. Combining an API with a pharmaceutically acceptable agent in this guest/host manner has

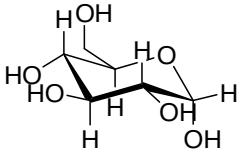
become an increasingly attractive route for developing pharmaceutical products. For example, co-crystallisation offers an alternative when salt screening is either unsuccessful or impossible (due to lack of ionisation sites) to improve the physical properties of a drug. Furthermore, exploring the co-crystallisation potential around an API increases the intellectual property protection over a particular drug product; thus, reducing the risk of costly litigation and market erosion. A recent development in the field has not only seen co-crystallisation as an alternative to salt studies, but has also seen its combination with salts to yield co-crystals of salts [14]. Co-crystals of API with common pharmaceutical excipients become very important [15,16].

Due to the above mentioned facts some experiments were designed to prepare co-crystals of ibandronate as a base for possible super generics. In the present study various mixtures of ibandronate and excipients (more hydrophobic adducts) in different ratios and under various conditions were prepared. All the prepared mixtures (solid compounds) and/or new entities were characterized by means of the Fourier transform near-infrared (FT-NIR) spectroscopy. Potential new co-crystals (new entities with predicted higher lipophilicity) were investigated for their absorption by means of experiments using the parallel artificial membrane permeation assay (PAMPA).

RESULTS AND DISCUSSION

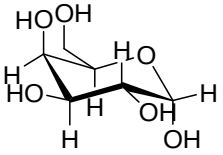
Various excipients and/or pharmaceutically acceptable agents were evaluated as potential counterions: α-D-glucose, α-D-mannose, α-D-galactose, methyl-α-D-glucopyranoside, methyl-β-D-galactopyranoside, methyl-α-D-mannopyranoside, phenyl-β-D-glucopyranoside, phenyl-β-D-galactopyranoside. All studied excipients are illustrated in Figure 2. The evaluated samples were prepared by means of dissolution of ibandronate and excipient and subsequent reverse obtaining of solid compounds that were characterized using the FT-NIR spectroscopy (diffuse reflectance method, DRIFT).

Figure 2. Excipients and pharmaceutically acceptable agents evaluated as potential counterions.

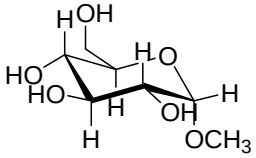


α-D-glucose

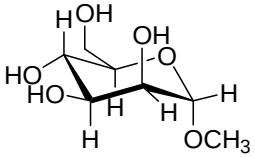
α-D-mannose



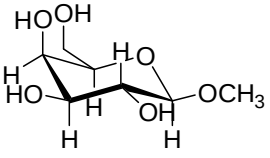
α-D-galactose



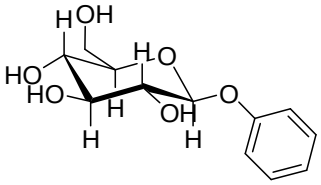
methyl-α-D-glucopyranoside



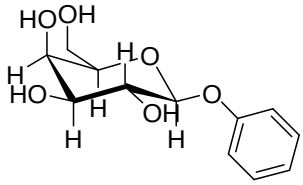
methyl-α-D-mannopyranoside



methyl-β-D-galactopyranoside



phenyl-β-D-glucopyranoside



phenyl-β-D-galactopyranoside

Optical sensing methods represent an important methodology in the modern analytical chemistry. The Fourier transform near-infrared (FT-NIR) spectroscopy is a fast and non-destructive modern analytical technique that offers many advantages for a broad range of applications, e.g. in pharmaceutical development and quality control and process analytical technology. A near-infrared spectral region (1100-2500 nm) is used for the analytical determination of chemical parameters in multiple matrices and industrial products. This has been due to the specific characteristics of this spectral region, the availability of modern instrumentation and the development of advanced chemometric approaches [17].
From all tested agents only phenyl-β-D-galactopyranoside generated co-crystals with ibandronate, see Figure 3. Samples of ibandronate mono-sodium salt : phenyl-β-D-galactopyranoside, in ratios 1:1 and 1:3 were prepared, see Figure 1. Absorption of both samples (co-crystals in ratios 1:1 and 1:3) were investigated using PAMPA experiments.

Figure 3. NIR spectra of ibandronate mono-sodium salt, phenyl-β-D-galactopyranoside and spectrum of their potential co-crystal.

Many low molecular weight drugs are absorbed through passive (or partially passive transport). Assays using artificial membranes, such as PAMPA, can be used as an alternative approach to assess *in vitro* transcellular passive permeation [13].
The permeability of ibandronate and both co-crystals 1:1 and 1:3 was tested. It is recommended for retaining interactions of both components in the solution to increase concentration of counterion in the applied mixture [16]. Therefore samples with addition of six fold quantity of phenyl-β-D-galactopyranoside to the co-crystal solution were also evaluated. The results of absorption study are shown in Table 1.

Table 1. Concentration [μg/mL] of ibandronate standard and co-crystal samples in acceptor solutions of PAMPA plates.

Compound	Concentration [μg/mL]
ibandronate mono-sodium salt	2.4
co-crystal 1:1	2.4
co-crystal 1:3	2.06
co-crystal 1:1 + phenyl-β-D-galactopyranoside (1:6)	<2
co-crystal 1:3 + phenyl-β-D-galactopyranoside (1:6)	<2

Unfortunately, the evaluated co-crystals of ibandronate and phenyl-β-D-galactopyranoside showed similar or relatively low absorption related to permeability of ibandronate API, see Table 1, which is in conflict with our expectation. According to the results in Table 1 it can be concluded that higher amount of phenyl-β-D-galactopyranoside probably blocked pores in artificial membrane and this caused a decrease in absorption.

EXPERIMENTAL

Generation of co-crystal

All excipients were purchased from Aldrich. All the evaluated samples were prepared by means of dissolution of ibandronate and excipient in aqueous methanol in different ratios (1:1 and 1:3) and subsequent return obtaining of a solid compound. Near infrared spectra were recorded using a Smart Near-IR UpDrift™, Nicolet™ 6700 FT-IR Spectrometer (Thermo Scientific, U.S.A.). The spectra were obtained by accumulation of 128 scans with 4 cm^{-1} resolution in the region of 12800-4000 cm^{-1}. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation, U.S.A.) with direct injection into the APCI source (400 °C) in the positive mode.

PAMPA experiment

The PAMPA plate (BD Gentest, BD Biosciences Discovery Labware, Bedford, MA, U.S.A.) was warmed to laboratory temperature. Compounds of the mixtures were dissolved as much as possible. Standards (ibandronate, phenyl-β-D-galactoside) were dissolved in H_2O-HPLC – Mili-Q Grade. Samples were dissolved in 0.01 M HCl and than pH was adjusted by bicarbonate buffer to pH 6.0. When the sample was evaluated in excess of phenyl-β-D-galactopyranoside, the ratio 1:6 (co-crystal : phenyl-β-D-galactopyranoside) was applied. The donor solutions (300 μL) was put on the plate and incubated for 5 hours. A physiological solution (200 μL) with pH 7.4 was used as an acceptor solution. After the incubation time the acceptor solution was directly injected to the mass spectrometer. Each sample was evaluated in 5 wells. The results are summarized in Table 1.

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