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Preformulation investigation and challenges; salt formation, salt disproportionation and hepatic recirculation

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ABSTRACT

A compound, which is a selective peroxisome proliferator activated receptor (PPAR) agonist, was investigated. The aim of the presented studies was to evaluate the potential of the further development of the compound. Fundamental physicochemical properties and stability of the compound were characterized in solution by liquid chromatography and NMR and in solid-state by various techniques. The drug itself is a lipophilic acid with tendency to form aggregates in solution. The neutral form was only obtained in amorphous form with a glass-transition temperature of approximately 0 °C. The intrinsic solubility at room temperature was determined to 0.03 mg/mL. Chemical stability studies of the compound in aqueous solutions showed good stability for at least two weeks at room temperature, except at pH 1, where a slight degradation was already observed after one day. The chemical stability in the amorphous solid-state was investigated during a period of three months. At 25 °C/60% relative humidity (RH) and 40 °C/75% RH no significant degradation was observed. At 80 °C, however, some degradation was observed after four weeks and approximately 3% after three months. In an accelerated photostability study, degradation of approximately 4% was observed. Attempts to identify a crystalline form of the neutral compound were unsuccessful, however, salt formation with *tert*-butylamine, resulted in crystalline material. Results from stability tests of the presented crystalline salt form indicated improved chemical stability at conditions whereas the amorphous neutral form degraded. However, the salt form of the drug dissociated under certain conditions. The drug was administered both per oral and intravenously, as amorphous nanoparticles, to conscious dogs. Plasma profiles showed curves with secondary absorption peaks, indicating hepatic recirculation following both administration routes. A similar behavior was observed in rats after oral administration of a pH-adjusted solution. The observed double peaks in plasma exposure and the dissociation tendency of the salt form, were properties that contributed to make further development of the candidate drug challenging. Options for development of solid dosage forms of both amorphous and crystalline material of the compound are discussed.

1. Introduction

Prior to the development of a drug product, it is essential that certain fundamental physical and chemical properties of the drug molecule are determined. This information dictates many of the subsequent events and approaches in the formulation development. This first learning phase is known as preformulation. These early studies have a significant part to play in anticipating formulation problems and identifying logical paths in both liquid and solid dosage form development. One of the first steps in the preformulation work is to establish analytical methods to be able to measure solubility and follow stability of the compound (Sigfridsson et al., 2011, 2012a,b). In the development of pharmaceutical dosage forms, one of the common challenges is to assure acceptable stability. It is critical to identify any instability in pharmaceutical formulations as early as possible in the development process, either by using *in silico* predictive tools (internal or commercial available) or by simply performing the actual investigations. It is also important to investigate, and in some cases, improve, the solid-state properties of the compound to find a suitable, preferably crystalline material, for further development (Serajuddin, 2007; Merritt et al., 2013; Sigfridsson et al., 2011, 2012b). Another important area to consider is the properties that affect drug delivery. These properties can be summarized as the properties of the drug (*e.g.* physicochemical properties, solubility and stability), the used formulation (*e.g.* immediate or modified release) and the route of administration (*e.g.* oral or intravenously), which all affect the rate and/or the extent of drug absorbed (Sigfridsson et al., 2011, 2012a,b). One way to focus technology and product development efforts is to use a Target Product

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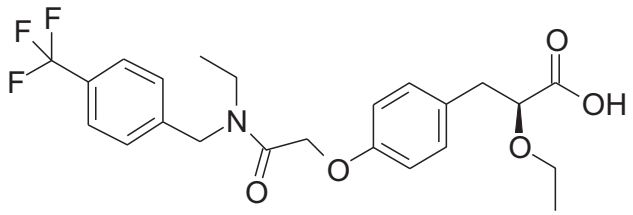

Fig. 1. The chemical structure of the API (S-configuration).

Profile (TPP) early in the project providing a clear and tangible vision and focus for the drug development program.

The investigated active pharmaceutical ingredient (API, Fig. 1), is a selective peroxisome proliferator activated receptor (PPAR) agonist. The tentative indication for the compound is correction of combined dyslipidemia with or without other manifestations of the metabolic syndrome. The anti-dyslipidemic effect and the potential direct anti-atherogenic actions are expected to reduce cardiovascular morbidity and mortality in the target patient population (Grygiel-Gorniak, 2014). The aim of the present work was to evaluate the potential to further development of a selected candidate drug. Initially, amorphous material was obtained, characterized and administered *in vivo* to conscious rats and dogs as pH-adjusted solutions and amorphous nanoparticles. Some early investigations of solid formulation approaches were performed. However, as soon as a crystalline salt form of the API was identified, the focus of the project shifted to the crystalline material and the initial characterizations performed on amorphous compound were repeated and extended. An API with a stable crystalline form is preferred from evaluation, development and commercial point of view. The crystalline material of the API was characterized regarding fundamental properties and stability, including photostability. A set of technical approaches was used to investigate the solid-state forms of the salt of the API. Among the techniques used were X-ray powder diffractometry (XRPD), differential scanning calorimetry (DSC), thermogravimetrical analysis (TGA), dynamic vapour sorption (DVS), Raman spectroscopy and Nuclear Magnetic Resonance (NMR).

2. Material and methods

2.1. Test compound

The API and a selection of salt forms of the API were synthesized and prepared at AstraZeneca R & D Gothenburg, Sweden.

2.2. Physicochemical measurements

The pK_a measurement was performed with the same methods as earlier described by Wan et al. (2003). Log P was calculated using ACD/Labs 6.00 C. 29 µM of the drug was dissolved in 1% acetonitrile in water and the absorbance at 220 nm was recorded (Varian Cary 50 UV/VIS spectrophotometer, Agilent Technologies, Santa Clara, CA) using 1 cm cuvette. The extinction coefficient was calculated using Beer-Lambert law. A KSV Sigma 70 tensiometer used a Wilhelmy plate to measure the surface tension forces and was bought from KSV Instruments Ltd., Helsinki, Finland.

2.3. Solubility measurements

The solubility of the compound in aqueous solutions or oils was determined at different pH, by adding an excess of drug into the solvent. The suspensions were stirred on a magnetic stirrer at 22 °C for 24 h, filtered (cut-off 0.22 µm, Millex-GV, PVDF, Millipore, Carrigtwohill, Co. Cork, Ireland) and the content of dissolved API was analyzed by HPLC as described below. The good solubility at intestinal pH, compared with the therapeutic dose levels, excluded the necessity to measure the solubility in real human intestinal fluid (HIF) and/or in (fasted or fed) simulated small intestinal fluids (FaSSIF and FeSSIF).

2.4. Salt screen

An initial salt screening was carried out whereby salts are formed by evaporation in a 96-well quartz micro plate and then analyzed for crystallinity by microscopy with crossed polarizers. Any crystals formed were analyzed for salt formation with a confocal Raman microscope. Salt screening was carried out for the following bases; $Mg(OH)_2$ (stoichiometry of acid:counter ion, 2:1), $Ca(OH)_2$ (2:1), KOH, ethylenediamine (2:1), *N*,*N*′-dibenzyl-ethylenediamine (1:1), *N*-Methyl-D-glucamine (1:1), triethanolamine (1:1), ethanolamine (1:1), piperazine (1:1), NaOH (1:1), diethanolamine (1:1), cholinehydroxide (1:1), LiOH (1:1), t-butyl amine (1:1), and tris(hydroxymethyl)aminomethane (TRIS, 1:1). The compound (73.6 mg) was dissolved in 10.0 mL methanol. 135.9 µL of the API solution (*i.e.* corresponding to 1 mg of API) was pipetted into 34 wells in a quartz 96-well micro plate. Counter-ion solution, in the stoichiometric ratios mentioned above, was then pipetted into the wells in duplicates (*i.e.* two wells for each salt). Four wells were left with free acid so that the salt screen was also a crystallization screen for the neutral form. The solvents were removed under a purge of nitrogen. Due to the very low degree of crystallinity obtained, the Raman spectra did not provide any useful information and was thus excluded. Different solvents (acetone, water, methanol/toluene or methanol/2-propanol) were then added to the salts, each solvent was removed by evaporation in air and/or nitrogen at room temperature.

With guidance from this initial salt screening, selected salts as well as the free acid form, were then scaled up and crystallization attempted under a wide range of conditions. The only form that was possible to isolate as crystalline after significant effort was tert-butylammonium salt (TBA, obtained by *e.g.* evaporation from acetone and acetone/water mixtures).

2.5. Equipment for solid-state measurements

X-ray Powder Diffractometry (XRPD) experiments were performed on a D8 Advance diffractometer (Bruxer AXS GmbH, Karlsruhe, Germany) with Bragg-Brentano geometry, equipped with a VÅNTEC-1 position sensitive detector (PSD). Differential Scanning Calorimetry (DSC) analysis was performed using a DSC Q1000 (TA Instruments, New Castle, USA). Thermogravimetrical Analysis (TGA) analysis was performed using a TGA Q500 (TA Instruments, New Castle, USA) with and without a linked Mass Spectrometer (MS) from Pfeiffer Vacuum GmbH. Water sorption measurements were carried out using a commercial instrument: Dynamic Vapour Sorption, DVS-1 (Scientific & Medical Products Ltd., Manchester, UK). The Raman measurements were performed with a Spectrum GX from Perkin Elmer (Waltham, Massachusetts, USA). The obtained solids from the salt screen were examined for crystallinity by microscopy using an Olympus microscope and crossed polarizer (Olympus with a Nikon SMZ1500, Shinjuku-ku, Tokyo, Japan). The crystalline samples were then analyzed for proof of salt formation using Raman microscopy (Labram HR 800 Raman microscope, Jobin Yvon/Horiba).

2.6. NMR

The ^{1}H NMR measurements were carried out on a Varian 500 MHz Inova instrument (Varian Inc., Palo Alto, CA) operating at 499.545 MHz. The NMR instrument was equipped with a 5 mm inverse detection probe. 5–10 mg of the samples were dissolved in 0.7 mL of MeOD. The temperature used was 25 °C. A typical ^{1}H NMR measurement was carried out by using a flip angle of 90° with an acquisition time of 4 s and a delay time of 56 s. The spectral width was at least between − 2 and 12 ppm referring to the solvent peak of MeOD (3.31 ppm) and 4 transients were