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ORIGINAL ARTICLE

Enantiomeric characterization and structure elucidation of Otamixaban

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Abstract Otamixaban is a potent (Ki = 0.5 nM) fXa inhibitor currently in late-stage clinical development at Sanofi for the management of acute coronary syndrome. Being unproductive in obtaining a suitable crystal of Otamixaban, the required enantiomeric characterization has been accomplished using vibrational circular dichroism (VCD) spectroscopy. Selected by a spectrum similarity index, the calculated spectra of several higher energy conformers were found to match well with the observed spectra. The characteristic IR bands of these conformers were also identified and attributed to the solvation effect. Combined with both the single crystal x-ray diffraction results for an intermediate and the proton NMR study, the absolute configuration of Otamixaban is unambiguously determined to be (*R*,*R*).

1. Introduction

As a potent (Ki = 0.5 nM) and selective Factor Xa (fXa) inhibitor, Otamixaban is in late-stage clinical development for the management of acute coronary syndrome [1]. Although its stereochemistry was established from the co-crystal structure with fXa [2], the absolute configuration (AC) of Otamixaban from a different synthetic route needs to be verified for both the regulatory requirement and quality control. The task is usually handled by the single crystal x-ray diffraction (scXRD) method [3]. However, our effort to obtain a suitable crystal of Otamixaban was unsuccessful. Since vibrational circular dichroism (VCD) spectroscopy [4–7] is an alternative method

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of AC determination, we attempted to explore this approach for Otamixaban.

The VCD method is based on the model-observation agreement. When a simulated spectrum of a model matches the experimental spectrum of a sample, the AC of the model is assigned to the sample. The challenges in Otamixaban AC determination are mainly due to the molecular size and flexibility, which affect both the modeling and experiment. With nine rotational bonds and a molecular weight of 446, Otamixaban is larger and more flexible than most molecules in published VCD studies [8–10]. To simplify the spectrum simulation of large molecules, Dunmire et al. [11] replaced ketoconzole using two small chiral fragments, of 308 and 304 molecular weight. This approximation, however, is not suitable for Otamixaban because its two chiral centers are directly bonded. While today's computing power is capable of the spectrum calculation for the whole Otamixaban, handling the sheer volume of spectrum comparisons and providing the convincing evidence of AC are not trivial.

On the VCD experiment of large and flexible chiral molecules, the band broadening and low signal-to-noise ratio (S/N) were frequently observed due to an increasingly larger number of fundamentals in the VCD sensitive region (1100–1500 cm^{-1}) and multiple populated conformers. As a result, a fewer band-to-band matches can be established between a model and observed spectrum. Because both the conformation and configuration can affect the number and sign of VCD bands, an AC assignment for such a molecule is often questionable without an error estimating.

To overcome the difficulties in the manual spectrum comparison, we [12] developed a VCD spectrum similarity index, S_V, which has been used to select the best matched spectra from calculations. We further discovered that the S_V of unmatched spectra has a normal distribution with a mean value of ~0 and a standard deviation of ~0.1 [13]. This statistical characteristic can be used to eliminate wrong models and to provide a confidence estimate for an assigned AC. We used the same approach herein for Otamixaban. To provide an unbiased assessment, we assumed no prior knowledge about the chirality of the sample thereby investigating all possible configurations. In addition, IR and NMR were used to study the solution conformations of Otamixaban.

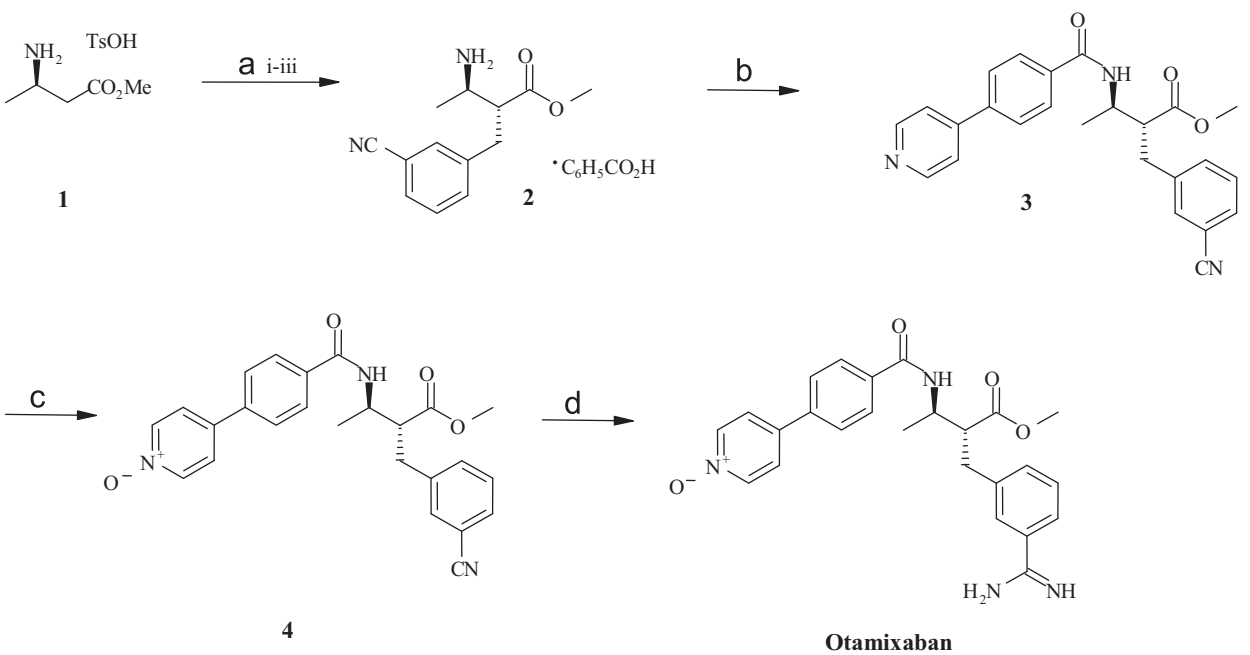
2. Experiment

2.1. Reagents and chemicals

The Otamixaban sample was prepared in our lab through the synthetic route described in Scheme 1.

2.2. IR and VCD data collection and processing

The experimentally observed IR and VCD spectra (4.6 mg Otamixaban dissolved in 0.15 mL DMSO-d_6) were measured by Biotools using ChiralIR-2X with Dual Pem. The spectra were acquired at the resolution of 4 cm^{-1} for 10 h with a path length of 100 μm. The solvent signal was subtracted from both spectra. The measured VCD spectra were corrected for baseline artifacts by subtracting the enantiomer spectra [10]. The conformation search was carried out using Maestro 8.5 (Schrödinger, LLC). In order to save computing resources and to identity the AC quickly, a coarse sampling with an energy cutoff of 5 kcal/mol (20.934 kJ/mol) and an RMSD of 2 Å were used to generate starting conformers for the subsequent density functional theory (DFT) calculations. After eliminating the unmatched stereoisomer, conformation searches with smaller RMSD of 0.5 Å were used to generate additional conformers. Similar to other studies [12,13], Gaussian 03 (Gaussian Inc.) [14] with 6-31G(d) basis set and PBEPBE functional were



Reagents and conditions: a) i. LiHMDS, THF, −20 °C; ii. 3-cyanobenzylbromide; iii. benzoic acid, water/toluene; b) i. aq. Na_2CO_3; ii. 4-Pyridin-4-yl-benzoic acid; iii. TBTU, NMM, DMF; c) MMPP, CH_2Cl_2/H_2O; d) i. HCl/MeOH; ii. NH_3.

Scheme 1 Synthesis route of Otamixaban.