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Notes

Synthesis and Structural Determination of Temocapril Sulfoxide Hydrochlorides

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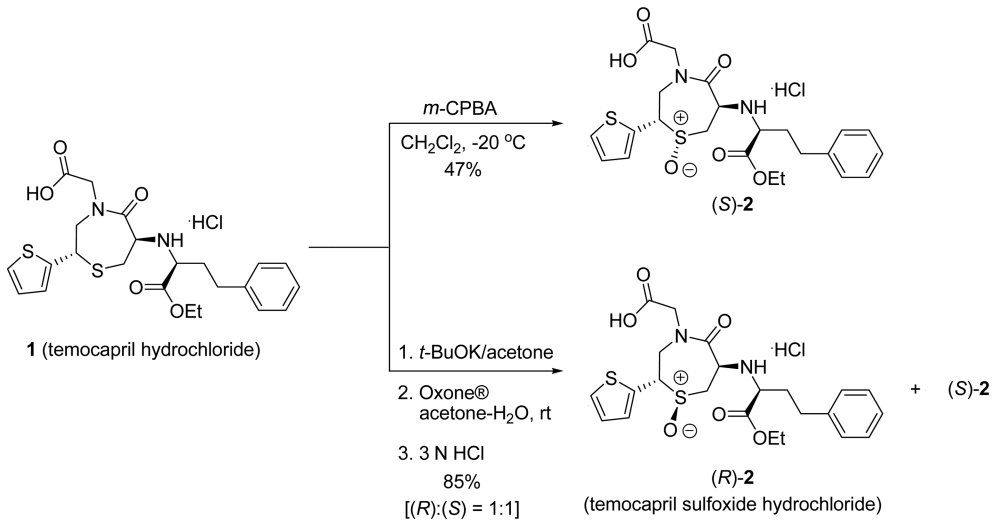
Impurity (or related substance) control in pharmaceutical products is a primary goal of drug development.[1] Stringent international regulatory requirements have been in place for several years as outlined in the International Conference on Harmonization (ICH) Guidelines Q3A (R), Q3B (R) and Q3C.[2-4] According to ICH guidelines, impurities associated with the manufacture of a drug substance, also known as an active pharmaceutical ingredient (API), are classified into the following categories: (1) organic impurities (process and drug-related); (2) inorganic impurities (3) residual solvents. Many potential impurities result from the API manufacturing process including starting materials, isomers, intermediates, reagents, solvents, catalysts and reaction by-products. These potential impurities should be investigated to determine process control mechanisms for their removal and the need for specification controls at appropriate points in the process. The suggested structures of the impurities can be synthesized and will provide the final evidence for their structures, previously determined by spectroscopic methods.[5,6] Therefore it is essential to know the structure of these impurities in the bulk drug in order to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. Isolation, identification and quantification of impurities help the pharmaceutical company to obtain a pure substance with less toxicity and safety in drug therapy.

Temocapril hydrochloride (**1**), (+)-[(2*S*,6*R*)-6-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetic acid hydrochloride, is a prodrug of an angiotensin-converting enzyme (ACE) inhibitor, temocaprilat.[7] This API has been originally developed by Sankyo and sold as a brand name of Acecol® for the treatment of hypertension since 1994 by Nippon Boehringer Ingelheim. The aim of this study is to synthesize and determine the structural characterization of temocapril sulfoxide hydrochloride (**2**), temocapril hydrochloride-related substance (or impurity) which was detected as an impurity during the bulk manufacturing process.

Temocapril sulfoxide hydrochloride (**2**) can be easily synthesized from the oxidation of temocapril hydrochloride (**1**) with oxidizing agent such as $KMnO_4$, H_2O_2, *m*-CPBA or Oxone®. First, the oxidation of compound **1** with *m*-CPBA at −20 °C provided the oxidized compound **2** as a single stereoisomer on TLC among two possible diastereoisomers due to the new chiral center of sulfur atom in compound **2**.



Scheme 1. Synthesis of temocapril sulfoxide hydrochlorides (**2**).

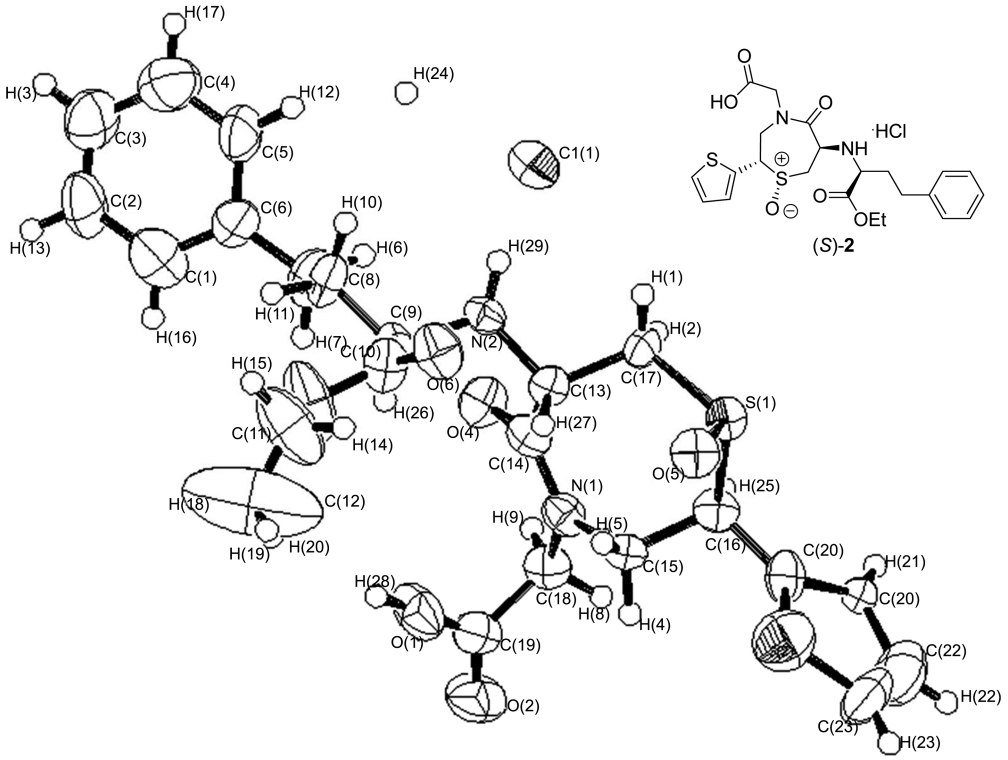


Figure 1. X-ray crystallographic structure of (*S*)-temocapril sulfoxide hydrochloride (**2**).

With this result in hands, the oxidation of free basic compound **1** with Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) at room temperature provided the oxidized compound **2** as two diastereoisomeric mixture on TLC and NMR spectrum, which could not be separated in spite of the application of appropriate separation techniques as shown in Scheme 1.

The structure of single stereoisomer **2** coming from the oxidation with *m*-CPBA was identified using ^{1}H NMR, ^{13}C NMR (including 90-DEPT and 135-DEPT), HH-COSY, HMQC, HMBC, and MS (FAB+) compared to those data of compound **1**. All NMR data and their interpretations were provided as Supporting Information.[8] With respect to mass spectroscopy, the total ion chromatogram for compound **2** showed an $[M+H]^+$ ion at *m/z* 493 compared to an $[M+H]^+$ ion for compound **1** at *m/z* 477, indicating the presence of sulfoxide group in product **2**. Because the absolute configuration of sulfoxide group in compound **2** cannot be determined solely using NMR spectrometry, the single stereoisomer **2** was also subjected to X-ray crystallographic characterization and finally identified to be (*S*)-configuration on sulfur atom as shown in Figure 1. The presence of (*R*)-form in diastereomeric mixture **2** from the oxidation reaction with Oxone® was identified using ^{1}H NMR, ^{13}C NMR, and LC-MS [C_{18} column and eluent: *n*-butanol-acetic acid-water (4:1:1)] compared to those data of pure (*S*)-**2**. With respect to LC-MS of diastereomeric mixture **2**, in particular, (*S*)-**2** and (*R*)-**2** showed the same $[M+H]^+$ ion at *m/z* 493 at 10.63 and 11.37 min (retention time), respectively.

The reason for this different stereochemical result depending on the oxidizing agent used is probably steric: Considering the energy-minimized stable 3D-conformation of temocapril hydrochloride (**1**) as shown in Figure 2, the top face of 7-membered ring was more hindered than its bottom face and thus less accessible to the bulky *m*-CPBA in spite of its plausible hydrogen bond with ammonium ion, affording (*S*)-**2** *via* the attack on bottom face. In the case of Oxone®, this small inorganic reagent can have easy access to both faces of 7-membered ring after the neutralization of ammonium salt, providing the almost same ratio of two diastereoisomers (**2**).

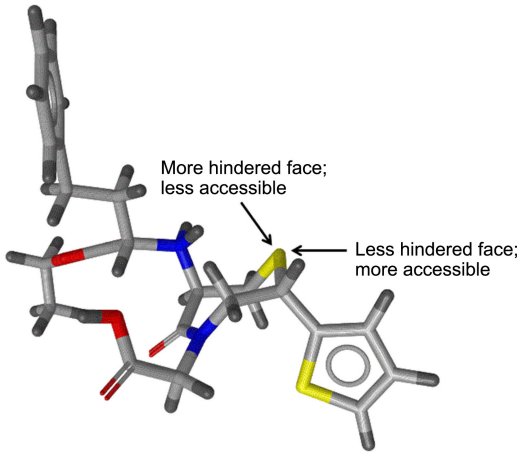


Figure 2. Energy-minimized 3D-conformation of temocapril hydrochloride (**1**). Figure was made using LigandScout.

In conclusion, impurity profiling is very important during the synthesis of drug substances and manufacture of dosage forms. In the present study, each diasteromeric temocapril

sulfoxide hydrochloride [(*S*)-**2** and (*R*)-**2**], temocapril hydrochloride-related substance (impurity), was prepared through the oxidation with *m*-CPBA and Oxone, respectively. The structure and absolute configuration of pure (*S*)-**2** was identified by using NMR, FAB-MS and X-ray crystallography. In the case of (*R*)-**2**, it was obtained as only diastereomeric mixture but its retention time was confirmed for the future qualitative analysis. These overall results would help the pharmaceutical company to prepare the impurity profiling of temocapril hydrochloride as API of antihypertension drug.

Experimental Section

General Methods. All NMR spectra were recorded on Bruker Advance 400 (400 MHz). Chemical shifts are expressed in ppm using residual $CDCl_3$ as reference. LC-Mass spectra were recorded on VG Biotech platform. All measurements for X-ray crystallographic structure were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Mo-Kα radiation. Energy-minimized 3D-conformation of temocapril hydrochloride (**1**) was made using LigandScout program.

Synthesis of (*S*)-Temocapril Sulfoxide Hydrochloride (2). To a stirred suspension of temocapril·HCl (**1**, 206 mg, 0.40 mmol) in 10 mL of CH_2Cl_2 was added *m*-CPBA (420 mg, 2.43 mmol) at –20 °C. The reaction mixture was stirred for 2 h at the same temperature and allowed to warm to room temperature after the complete reaction on TLC. The resultant solid was filtered and recrystallized with MeOH/IPA to afford the target compound (100 mg, 47%) as a pure and white solid: MS (FAB+) *m/z*: 493 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.62 (1H, d, J = 5.2 Hz), 7.37-7.25 (5H, m), 7.23-7.05 (2H, m), 5.29 (1H, m), 4.82-4.73 (2H, m), 4.44 and 4.17 (ABq, 1H, d, J = 17.5 Hz), 4.30-4.16 (2H, m), 4.08 (1H, t, J = 5.0 Hz), 3.80 (1H, d, J =14.5 Hz), 3.56 (1H, d, J = 15.6 Hz), 3.45 (1H, dd, J = 10.7, 14.5 Hz), 2.82 (1H, m), 2.61 (1H, m), 2.26-2.23 (2H, m), 1.28 (3H, t, J = 7.1 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.44, 169.07, 168.27, 140.15, 136.77, 128.36, 128.22, 127.11, 127.07, 126.70, 126.13, 61.92, 58.10, 55.05, 50.73, 46.52, 44.37, 43.39, 31.13, 30.14, 13.78.

Synthesis of Diastereomeric Mixture of Temocapril Sulfoxide Hydrochlorides [(*S*)- and (*R*)-(2)]. A suspension of temocapril·HCl (**1**, 516 mg, 1.01 mmol) in 50 mL of acetone was treated with potassium *t*-butoxide (226 mg, 2.02 mmol) to provide a clear solution, which was diluted with 30 mL of distilled water. The diluted solution was treated with Oxone® ($2KHSO_5KHSO_4K_2SO_4$; 309 mg; 1.01 mmol of $KHSO_5$) at room temperature and further stirred overnight. After the removal of all solvent, the residue was diluted with 30 mL of MeOH and the resultant precipitate was filtered out. The filtrate was concentrated, diluted with 20 mL of distilled water, and adjusted to pH 4 with 3 N HCl solution. The acidified solution was sonificated and concentrated under reduced pressure to afford a mixture (454 mg, 85% recovered yield), which was identified to consist of temocapril HCl (**1**), (*R*)- and (*S*)-temocapril sulfoxide HCl (**2**) with a ratio of 0.35:1:1 on LC-MS and ^{1}H NMR based on H-1 chemical shifts.

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Reference and Notes

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