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Research Paper

Synthesis and Characterization of Unknown Impurities in Nizatidine

Gaddam Naveen Chandra Reddy,^{a,b} Jyothirmayi Naram,^a Rakeshwar Bandichhor,^a Padi Pratap Reddy,^c P. K. Dubey,^b and Dinesh S. Bhalerao,^{a*}

^aCenter of Excellence, Integrated Product Development, Innovation Plaza, Dr. Reddy's Laboratories Ltd., Bachupalli, Qutubullapur, R. R. Dist. 500 072 Andhra Pradesh, India.

^bCollege of Engineering, JNT University, Kukatpally, Hyderabad-500072, Andhra Pradesh, India.

^cResearch & Development Center, Macleods Pharmaceutical Ltd, G/2, Mahakali Caves Road, Shanthi Nagar, Andheri East, Mumbai 400093.

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Abstract: Three impurities of Nizatidine, impurity (1), impurity (2) and impurity (3) were identified and synthesized in formulations during preparation of oral solution as well as in stability studies.

Introduction

Nizatidine is a oral histamine H₂-receptor antagonist similar to Cimetidine, Famotidine and Ranitidine. Nizatidine inhibits stomach acid production, and commonly used in the treatment of peptic ulcer disease (PUD) and gastroesophageal reflux disease (GERD). Nizatidine is available as the prescription drug and as a nonprescription product for relief of heartburn, acid indigestion and sour stomach. It was developed by Eli Lilly and is marketed under the brand names Tazac and Axid.

Certain preparations of nizatidine are now available over the counter in various countries including the United States.

Corresponding Author*: Fax: +91 40 44346285
E-mail address: dineshb@drreddys.com (Dinesh Bhalerao)

Nizatidine has been used experimentally to control weight gain associated with some antipsychotic medication.^[1]

Nizatidine was developed by Eli Lilly, and was first marketed in 1987. It is considered to be equipotent with ranitidine and differs by the substitution of a thiazole-ring in place of the furan-ring in ranitidine. In September 2000 Eli Lilly announced they would sell the sales and marketing rights for Axid (Nizatidine) to Reliant Pharmaceuticals.^[2] Subsequently, Reliant developed the oral solution of Axid, marketing this in 2004, after gaining approval from the U.S. Food and Drug Administration (FDA).^[3] However, a year later they sold rights of the Axid Oral Solution (including the issued patent US6,930,119 protecting the product) to Braintree Laboratories.^[4] Nizatidine

proved to be the last new histamine H₂-receptor antagonist introduced prior to the advent of proton pump inhibitors.

Active Pharmaceutical Ingredients (API) that are taken for human consumption have to follow certain guidelines outlined by the regulatory authorities such as FDA and ICH. Though purity of the API is an important criterion for accepting a particular drug for the human use, it is very much critical that the impurities are also in specified limits. Recently we published a paper for the synthesis of N-2-mono-N-desmethyl Nizatidine which is a metabolite impurity.^[5] During the preparation of formulation as well as stability studies of Nizatidine, three new peaks were identified during HPLC analysis which were in border or above acceptable ICH limits at respective retention time. To identify these impurities, fractions of these impurities were collected during HPLC analysis at respective retention time and submitted for mass and HRMS. Based on mass and HRMS data following structures^[6] were proposed.

Results and Discussion

Impurity (1) (Thiazole derivative)

To compare ES-MS-MS for impurity (1) and nizatidine, data has collected and found that m/z 155, 215, 232 and 271 are the common fragments in both impurity (1) and nizatidine. This fragmentation pattern shows that part of impurity (1) structure is similar to that of nizatidine. It indicates that to the structure of nizatidine, (2-((dimethylamino)methyl)thiazol-4-yl)methyl cation moiety has been attached to N-methyl nitrogen (HN-CH₃) or olefinic carbon.

To identify at which position (2-((dimethylamino)methyl)thiazol-4-

yl)methyl cation moiety is attached to nizatidine in impurity (1), deuterium exchanged MS studies were conducted to know how many exchangeable protons were present in impurity (1) by dissolving the sample in D₂O and CD₃OD. Before the analysis, this sample was kept at room temperature for an hour, for complete exchange of exchangeable protons with deuterium atoms. The sample was infused into mass spectrometer and the mass number obtained is m/z 489. The extra mass units can be attributed to two exchangeable protons in impurity (1) which indicates that (2-((dimethylamino)methyl)thiazol-4-yl)methyl cation moiety has attached to olefinic carbon and two -N-H protons are free for exchange.

The ¹H NMR spectra shows two aromatic singlet peaks at δ 8.26 and 7.7ppm which indicates that impurity (1) structure consists of two thiazole rings. The ¹H NMR spectra shows twelve protons as multiplet at δ 2.98-2.83ppm which indicates that impurity X structure consists of two N,N-dimethylamino groups(-N(CH₃)₂). From the Mass, HRMS and NMR data, the structure of the impurity (1) is confirmed as (E)-3-(2-((dimethylamino)methyl)thiazol-4-yl)-N-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethyl)-N-methyl-2-nitroprop-1-ene-1,1-diamine and its molecular formula is C₁₉H₃₁N₇O₂S₃. The HPLC purity of impurity 1 is 91.5%.

Impurity (2) (Cinnamaldehyde adduct)

The HPLC analysis of Impurity (2) showed 91.5% of pure compound after column chromatography. The positive electrospray Ionization (ESI) mass spectrum of Impurity (2) displayed molecular ion peaks at m/z 464 and 486 corresponding to the adduct ions (M+H)⁺ and (M+Na)⁺. The adduct ions confirmed the protonated molecular ion to

be m/z 464. As per HRMS data, the impurity (2) show mass of 464 and molecular formula of $C_{21}H_{30}N_5O_3S_2$ in positive HRMS and shows mass of 462 and molecular formula of $C_{21}H_{28}N_5O_3S_2$ in negative HRMS. The Nizatidine molecular formula is $C_{12}H_{21}N_5O_2S_2$. When compared with nizatidine, the impurity (2) molecular formula has nine carbons, eight hydrogens and one oxygen atom extra (C_9H_8O) which indicates that molecular formula C_9H_8O might be cinnamaldehyde because the cinnamaldehyde is present in bubble gum flavour that has been used during formulations. The impurity (2) is forming during the preparation of formulations and stability studies.

The 1H NMR data of impurity (2) when compared with nizatidine shows the absence of one methine proton (6.5ppm) and one exchangeable proton. These protons were attributed to the nitroethene methine and methyl attached to exchangeable proton. There are five aromatic protons extra in impurity (2) which indicates that one phenyl moiety might be present. Four aliphatic protons (one methine proton of tetrahydropyridin-2-ol ring is attached to phenyl group at 4.45ppm, one methine proton of tetrahydropyridin-2-ol ring is attached to hydroxyl group at 4.3ppm, and one methylene protons gives two peaks Ha at 2.0 and Hb at 2.2ppm) and one exchangeable proton (OH group) at 6.3ppm were observed between 2.0 to 6.3ppm in impurity (2). The remaining part of the structure of impurity (2) is similar to that of nizatidine.

As per IR, the Impurity (2) shows the presence of nitro group, -N-O stretching at 1575 and 1350 cm^{-1} and presence of hydroxyl group, -OH stretching at 3720 and cm^{-1} .

From the Mass, IR, HRMS and NMR data, the impurity (2) is confirmed as 6-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethylamino)-1-methyl-5-nitro-4-phenyl-1,2,3,4-tetrahydropyridin-2-ol and molecular formula is $C_{21}H_{30}N_5O_3S_2$.

Impurity (3) (N-Methyl pyridinium adduct)

The positive electrospray ionization (ESI) mass spectrum of Impurity (3) displayed molecular ion peak at m/z 399. As per HRMS data, the impurity (3) does not show negative ES-MS peaks which indicate that the molecular ion obtained was positively charged. As per HRMS, the molecular formula proposed for impurity (3) is $C_{21}H_{27}N_4S_2^+$. When compared with nizatidine impurity (3), this impurity has 64 mass units less, which corresponds to molecular formula H_2NO_3 which indicates the absence of nitro and hydroxyl groups in impurity (3).

For the identification of the number of exchangeable protons in impurity (3), the deuterium exchanged MS studies were performed by dissolving the sample in D_2O and CD_3OD . This sample was kept at room temperature for an hour, before the analysis, for complete exchange of exchangeable protons with deuterium atoms. The sample was infused into mass spectrometer and the mass number found to be m/z at 400.17. The additional one mass unit can be attributed to one exchangeable proton.

The 1H NMR spectrum of Nizatidine impurity (3) was compared with impurity (2) and found little differences. The 1H NMR spectrum of Nizatidine impurity (3) shows the absence of aliphatic peaks at 2.0 ppm (methylene Ha), 2.2ppm (methylene Hb), 4.3ppm (methine attached to OH carbon), 4.45ppm (methine attached to the pyridine

carbon to which phenyl group is attached) and hydroxyl peak at 6.3ppm. ^1H NMR shows additional three aromatic peaks at 7.1, 6.9 and 7.8 ppm as compared with impurity (2).

As per IR, the Impurity (3) shows the absence of nitro group, (-N-O stretching absent at 1575 and 1350 cm^{-1}) and absence of hydroxyl group (-OH stretching absent at 3720 cm^{-1}).

During the synthesis of Nizatidine impurity (3) or N-Methyl pyridinium adduct with Nizatidine, initially impurity (2) or Cinamaldehyde adduct also forms along with impurity (3) under heating.

From the Mass, IR, HRMS and NMR data, the impurity (3) is confirmed as 2-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethylamino)-1-methyl-4-phenylpyridinium and its molecular formula is $\text{C}_{21}\text{H}_{27}\text{N}_4\text{S}_2^+$.

Experimental

General Methods and Materials

The ^1H NMR spectra were recorded in DMSO-d_6 , D_2O , CDCl_3 at 400 MHz, 500 MHz on a Varian Mercury Plus NMR spectrometer and Varian Unity INOVA FT-NMR spectrometer. The ^1H NMR chemical shift values were reported on the δ scale in ppm, relative to TMS ($\delta=0.00\text{ppm}$) and the chemical shift values were reported relative to CDCl_3 ($\delta=77.00\text{ppm}$) (Cambridge Isotopic Labs, USA) and DMSO-d_6 ($\delta=39.50\text{ppm}$) in ^{13}C as internal standards, respectively. The ESI mass spectrums of impurities were recorded on Shimadzu 2010EV LCMS system. The HRMS studies were performed in high resolution mode on Waters LCT Premier XE. The IR spectra of impurity samples were recorded in the solid

state as KBr pellet using FT-IR (Perkin Elmer, spectrum one) Spectrophotometer. The solvents and reagents were used without any purification.

(E)-3-(2-((dimethylamino)methyl)thiazol-4-yl)-N-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethyl)-N-methyl-2-nitroprop-1-ene-1,1-diamine (Impurity (1) or Nizatidine thiazole derivative)

To a solution of 4-chloromethyl-2-dimethylaminomethyl thiazole hydrochloride (10.0 g, 44.0 mmol) in methanol (100.0 mL) was added a Nizatidine (14.0 g, 42.0 mmol) and triethylamine (15.3 mL, 11.03 mmol). The solution was heated at 60-70°C for 3.0 hrs. Then stirred reaction mixture at 25-35°C for overnight. Distilled off methanol under reduced pressure and dissolved the obtained residue in water (100.0 mL) and washed the aqueous layer with chloroform (7×200.0 mL). Distilled off aqueous layer at 50-60°C under reduced pressure. Added toluene (2 × 100.0 mL) and distilled off reaction mixture at 50-60°C. Further Nizatidine impurity (1) was purified by column chromatography (80% $\text{CH}_3\text{OH}/\text{CHCl}_3$). The HPLC purity of impurity (1) is 91.5%.

The positive electrospray Ionization (ESI) mass spectrum of Nizatidine impurity (3) displayed molecular ion peak at m/z 486; HRMS data (TOF MS ES+) in positive mode shows molecular ion peak at 486 and corresponds the molecular formula of $\text{C}_{19}\text{H}_{32}\text{N}_7\text{O}_2\text{S}_3$. ^1H NMR (Varian 500 MHz, D_2O): δ 8.26 (s, 1H), 7.7 (s, 1H), 3.99 (s, 2H), 3.44-3.23 (m, 2H ((CH_3)₂-N- CH_2 -), 2H ((CH_3)₂-N- CH_2 -), 2H ($\text{H}_3\text{CHN-C}=\text{C-CH}_2$ -), 2H (-S- CH_2 - CH_2 -NH-), 3H ($\text{H}_3\text{CHN-C}=\text{C-CH}_2$ -) and 2H (-S- CH_2 - CH_2 -NH-)) and 2.98-2.83 (m, 6H((CH_3)₂-N- CH_2 -) and 6H((CH_3)₂-N- CH_2 -); IR : 3391

cm⁻¹ (amine N-H Stretch), 1623 cm⁻¹ (aromatic C=C Stretch), 1575 and 1394 cm⁻¹ (nitro N-O Stretch).

6-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethylamino)-1-methyl-5-nitro-4-phenyl-1,2,3,4-tetrahydropyridin-2-ol (Impurity (2) or Nizatidine Cinnamaldehyde Adduct)

A mixture of Nizatidine (5.5g, 16.59 mmol) and Cinnamaldehyde (12.0mL, 95.3 mmol) was stirred at room temperature. The progress of the reaction was monitored by TLC and HPLC. After completion of reaction compound was purified by column chromatography (5% MeOH / CHCl₃) which gives 0.5g of Nizatidine cinnamaldehyde adduct (Impurity (2)) with 91.5% HPLC purity.

The positive electrospray Ionization (ESI) mass spectrum of Nizatidine cinnamaldehyde adduct impurity (Impurity (2)) displayed molecular ion peak at m/z 464 and 486[M+Na]⁺; HRMS data (TOF MS ES+) in positive mode shows molecular ion peak at 464 and corresponds the molecular formula of C₂₁H₃₀N₅O₃S₂. HRMS data (TOF MS ES-) in negative mode shows molecular ion peak at 462 and corresponds the molecular formula of C₂₁H₂₈N₅O₃S₂; ¹H NMR (Varian 400 MHz, DMSO-d₆): δ 11.1(br, 1H(NH)), 7.4-7.1 (m, 6H), 6.3 (d, 1H(OH)), 4.45(t, 1H (-CHOH-CH₂-CH-Ph)), 4.3(m, 1H(CHOH-CH₂-CH-Ph)), 3.9-3.5(m, 2H(-CH₂-S-CH₂-CH₂-NH-), 2H((CH₃)₂-CH₂-) and 2H(-S-CH₂-CH₂-NH-), 3.0 (s, 3H(N-CH₃)), 2.8(m, 2H(-S-CH₂-CH₂-NH-)), 2.3 (s, 6H((CH₃)₂-N-CH₂-), 2.2 (m, 1H(-CHOH-CH-H-CH-Ph)) and 2.0 (m, 1H(-CHOH-CH-H-CH-Ph)); IR : 3758 and 3720 (hydroxyl -OH Stretch), 2950-2850 cm⁻¹ (alkyl C-H stretch), 1575 and 1350cm⁻¹ (nitro N-O stretch).

2-(2-((2-((dimethylamino)methyl)thiazol-4-yl)methylthio)ethylamino)-1-methyl-4-phenylpyridinium (Impurity (3) or Nizatidine N-methyl Pyridinium adduct)

A solution of nizatidine (5.0 g, 15.08 mmol) and cinnamaldehyde (2.1 g, 15.9 mmol) in isopropanol (50.0 mL) was refluxed and progress of the reaction was monitored by TLC and HPLC. After completion of reaction solvent was evaporated by lyophilisation (Freeze drying) and obtained 1.5g of Nizatidine N-methyl pyridinium adduct or Impurity (3) with 92.0% pure.

The positive electrospray Ionization (ESI) mass spectrum of Nizatidine N-methyl pyridinium adduct impurity or impurity (3) displayed molecular ion peak at m/z 399. HRMS has done in positive and negative mode. But it doesn't showed negative ES-MS peaks which indicate that the molecular ion formed was positively charged. The established molecular formula of impurity (3) is C₂₁H₂₇N₄S₂⁺. ¹H NMR (Varian 500 MHz, ¹H NMR CDCl₃): δ 7.8-6.9 (m, 9H), 4.0 (s, 3H (N-CH₃)), 3.9-3.6(m, 2H(-CH₂-S-CH₂-CH₂-NH-), 2H((CH₃)₂-CH₂-) and 2H(-S-CH₂-CH₂-NH-)), 2.9(m, 2H(-S-CH₂-CH₂-NH-)), 2.3 (s, 6H((CH₃)₂-N-CH₂-); IR : 3062- 3014 cm⁻¹ (alkenyl C-H stretch), 2928-2856 cm⁻¹ (alkyl C-H stretch).

Conclusion

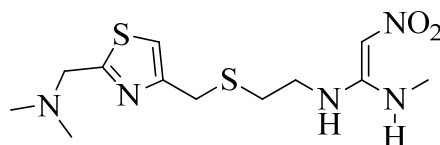
The three impurities of Nizatidine (Impurity (1), Impurity (2) and Impurity (3)) were synthesized and characterized using HPLC, Preparative HPLC, IR, Mass and NMR techniques.

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Nizatidine

Fig. 1 Structure of Nizatidine

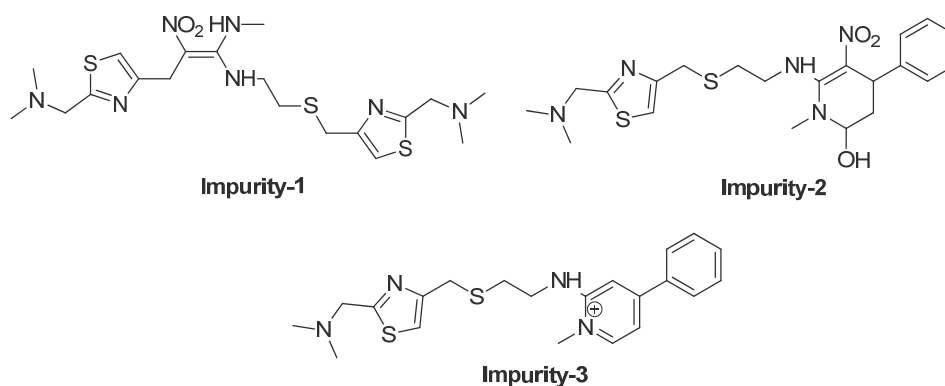
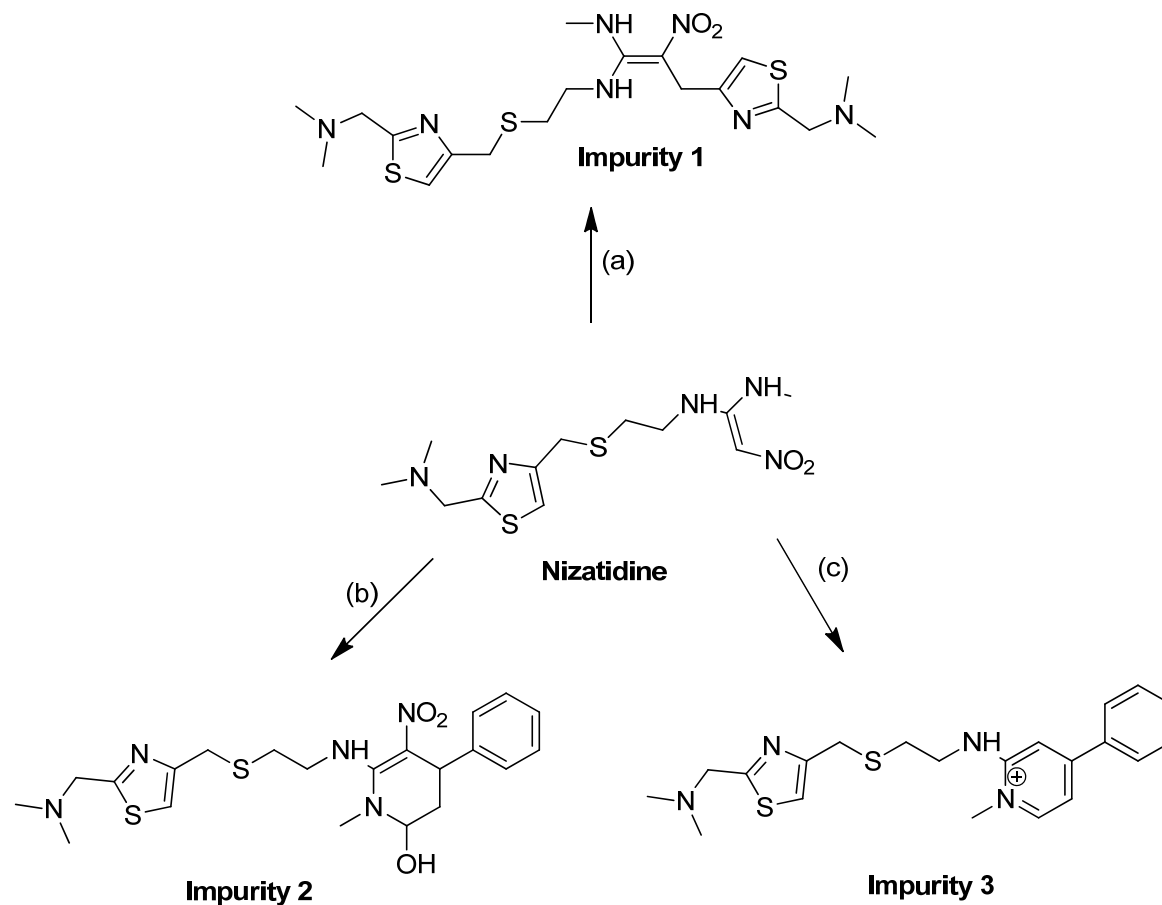


Figure 2: Structure of impurities



Scheme-1. Reagents and conditions: (a) 4-chloromethyl-2-dimethylaminomethyl thiazole hydrochloride, MeOH, TEA, 60-70°C; (b) Cinnamaldehyde, room temperature; (c) Cinnamaldehyde, IPA, 80-85°C.

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