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SYNTHESIS AND CHARACTERIZATION OF NOVEL AND POTENTIALN IMPURITIES OF DARIFENACIN, A POTENT MUSCARINIC M3 RECEPTOR ANTAGONIST

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ABSTRACT

Darifenacin Hydro bromide (Enablex) is a potent muscarinic M3 receptor antagonist, used for the treatment of overactive bladder with symptoms of urge urinary incontinence. During the large scale synthesis of darifenacin hydro bromide, four potent impurities were observed viz.., darifenacin acid, darifenacin desnitrile, darifenacin vinyl phenol, darifenacin ether. The present work describes the synthesis and characterization of these four impurities.

Keywords: LCMS, impurities, darifenacin, spectral characterization, Synthesis and silica column

INTRODUCTION

Darifenacin Hydro bromide (Enablex) is the generic name of *(S)*-2-{1-[2-(2,3-dihydrobenzofuran-5-yl) ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide hydro bromide, indicated for the treatment of overactive bladder[1]. Overactive bladder is used to describe a collection of urinary symptoms composed of urgency, with or without urge incontinence, usually with frequency and nocturia, in the absence of proven infection or other obvious pathology. As a selective antagonist of the M_3 receptor (the major subtype that modulates urinary bladder muscle contraction), darifenacin has a clinically significant effect on bladder function and control[2].

The presence of impurities in an active pharmaceutical ingredient (API) can have significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity profile of the API to be used in the manufacturing of a drug product. International conference on Harmonization (ICH) guidelines recommends identifying and characterizing all impurities that are present at a level of 0.10%[3]. In this context, a comprehensive study was undertaken to synthesize and characterize the following four impurities. Three of the four darifenacin impurities viz.., desnitrile **4**, ether **5** and vinyl phenol **6** impurities discussed here were not reported till date.

EXPERIMENTAL

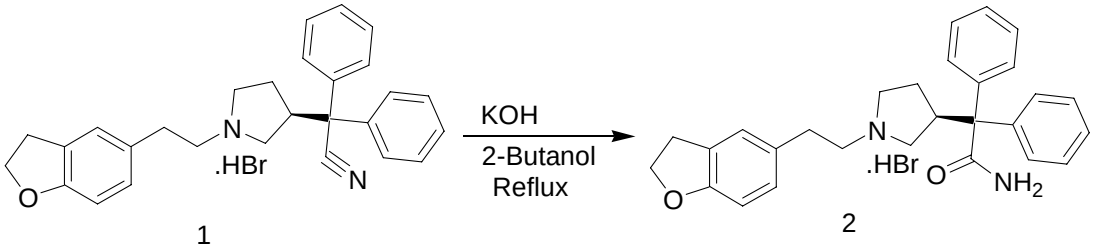
The ^{1}H-NMR spectra data were recorded at 200MHz on Varian and 400MHz on Varian, Gemini-2000, FT NMR spectrometers; the chemical shifts were reported in δ ppm relative to TMS. The infrared spectra were obtained using a Perkin-Elmer, Spectrum One Fourier Transform Infrared (FT-IR) spectrophotometer, with substances being pressed in KBr pellets. The mass analysis was performed on a AB-4000 Q-trap LC-MS=MS mass spectrometer. Solvent removal was accomplished by a rotary evaporator operating at house vacuum (40–50 Torr). The solvents and reagents were used without further purification.

General procedure for the synthesis of all the four impurities:

2-Butanol (225.0 mL) was added to a flask containing darifenacin freebase (15.0 g, 0.035 mol) and potassium hydroxide (98.6 g, 1.76 mol), and the mixture was stirred at 20-30°C for 1hour. Then the mixture was maintained at 100-105°C for 60 hours and the reaction mass cooled to ambient temperature. The suspension was stirred for 1 hour at 25-30°C and quenched with water 150mL fallowed by layer separation. The resultant organic layer was washed with 2×150 mL water and finally washed with 2.0% aqueous NaCl solution 50.0mL. The aqueous parts were extracted with 150 mL of ethyl acetate. The combined organic layer (2-Butanol + ethyl acetate) was dried over Na_2SO_4 and concentrated on a rotavapour at 60°C under reduced pressure. The resultant residue having 10 to 20% of each impurity, when subjected to HPLC. The individual impurities were separated by flash column chromatography on silica gel by eluting with ethyl acetate-hexanes 1:1 and methanol–dichloro methane 10:0, 9:1 and 7:3 ratio's to get desnitrile 4, vinyl phenol 6, acid 3 and ether 5 respectively.

RESULTS AND DISCUSSION

Darifenacin was synthesized in the laboratory by the reported protocols as depicted in figure-1[4]. During the process development of darifenacin hydro bromide, four impurities were observed in the conversion of partial hydrolysis of nitrile to amide using potassium hydroxide and 2-butanol.



Scheme-1: Synthesis of darifenacin hydro bromide

The crude sample was analyzed by reverse phase HPLC showing four unknown impurities in the range of 0.3 to 0.8%. The same material was subjected to LC-MS analysis to get the molecular weights of impurities. Thus the molecular weights of four impurities were identified as 427, 383, 500 and 426 respectively. Out of four impurities two (m/z 427 and 383) were process related and the other two were (m/z 500 and 426) process degradents.

The first two impurities are formed due to the high alkalinity and the traces of water present in the reaction medium as a result causing the hydrolysis of amide followed by de-carboxylation and the other two were formed by unusual Chemistry. The m/z 500 is formed by reacting the secondary butoxide ion with $–CH_2$ of ether function and m/z 426 can be formed by the attack of hydroxide ion with $–CH_2$ of ether function fallowed by dehydration leads to vinyl phenol as shown in the below synthetic scheme **(Scheme-2).**

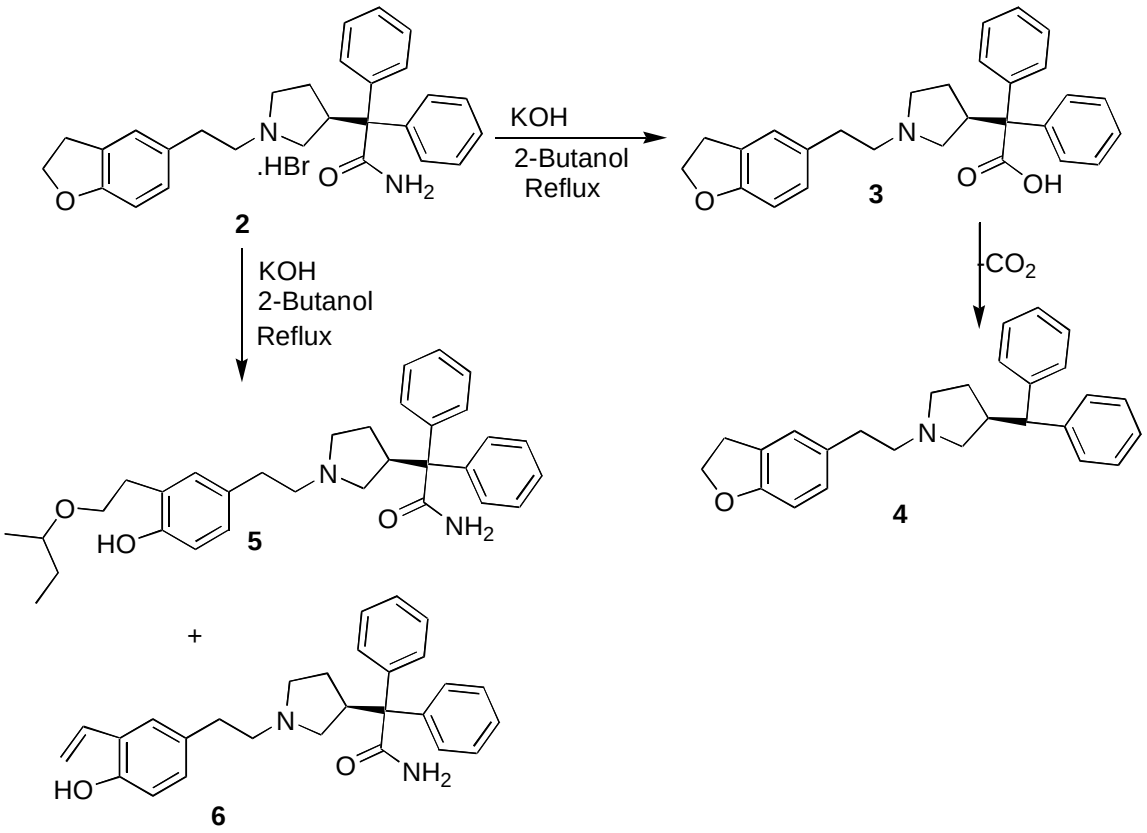
The above said impurities were prepared by reacting darifenacin freebase in high concentration of potassium hydroxide (50.0 equivalents) in 15 volumes of 2-butanol for 60 to 80 hours. The resultant crude product was subjected to HPLC, containing each 10 to 20% (%area) of all the four impurities. The residue was subjected to purification by column chromatography.

The ESI +ve ionization spectrum of all the four impurities i.e **3**, **4**, **5** and **6** displayed the protonated molecular ion at m/z= 428, 384, 501 and 427 respectively. The mass spectral fragmentation of all impurities gave single base peak at m/z 146 was attributed to corresponding 2,3-dihydrobenzofuran vinyl ion as depicted in figure-1.

The proton NMR spectrum of **3** didn't show the presence of two singlets between δ 5.0 to δ 6.0 ppm, inferring the absence of the amide group of darifenacin, suggests the absence of NH_2 function. The IR spectrum of **3** showed the broad peak at 3437 cm^{-1} indicates the presence of –OH function, -C=O stretching (1603 cm^{-1}). The ^{1}HNMR and IR data confirms the absence of $–NH_2$ and the presence of

carboxylic acid function in **3**, which is named as (S)-2-[1-[2-(2,3-dihydrobenzofuran-5-yl) ethyl]-3-pyrrolidinyl]-2,2-diphenylacetic acid (acid, **3**).

The proton NMR spectrum of **4** didn't show the presence of two singlets between δ 5.0 to δ 6.0 ppm, inferring the absence of the amide group of darifenacin and shown one doublet at δ 3.75 ppm belongs –C-H, which is linked to two phenyl groups. The IR spectrum of **4** was not showed the $–NH_2$ stretching at 3437 cm^{-1} and also C=O stretching of amide at ~1670 cm^{-1}. The ^{1}HNMR and IR data confirms the absence of C=O, $–NH_2$ and the presence of benzhydrol function and is confirmed as 3-benzhydryl-1-[2-(2,3-dihydro-benzofuran-5-yl)-ethyl]-pyrrolidine (desnitrile, **4**).



Scheme-2: Synthesis of darifenacin acid, desnitrile, ether and vinyl phenol

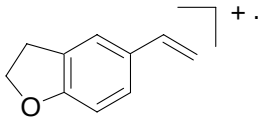


Fig.1: Main fragment of darifenacin acid, desnitrile, ether and vinyl phenol

The proton NMR spectrum of **5** displayed signal as (δ ppm): 5.5 (br, 2H of NH, D_2O exchangeable), 0.98 (t, 3H, CH_3), 1.15 (d, 3H, CH_3), 1.45(m, 2H, CH_2), 3.5 (s, 1H, -O-H of phenol), 4.1 (m, 1H, -O-CH), 4.7 (t, 2H, -O-CH_2) confirms the presence of secondary butoxy group and phenolic –OH function by the opening of ether linkage of dihydrobenzofuran ring system. The IR spectrum of **5** was showed the sharp

peak of $-NH_2$ stretching at 3470cm^{-1}, broad peak at 3378cm^{-1} of –OH, C-O stretching at 1217,1078cm^{-1} and C=O stretching of amide at 1674cm^{-1}. The ^{1}HNMR and IR data confirms the presence of secondary butoxy and phenolic -OH groups in **5** and hence, it is confirmed as 2-(1-{2-[3-(2-sec-Butoxy-ethyl)-4-hydroxy-phenyl]-ethyl}-pyrrolidin-3-yl)-2,2-diphenyl-acetamide (ether, **5**).

The proton NMR spectrum of **6** displayed signal as (δ ppm): 5.6 (br, 2H of NH, D_2O exchangeable), 5.28 (d, 1H, CH_2 of olephinic), 5.7 (d, 1H, CH_2 of olephinic), 6.7 (d, 1H, CH of olephinic), 3.5 (broad, 1H, -O-H of phenol), and the signals between δ 3.6 to 5.0 of O-CH_2 was not observed. This confirms the presence of vinyl group and phenolic –OH function by the opening of ether linkage of dihydrobenzofuran ring system. The IR spectrum of **6** was showed the very broad peak of $-NH_2$ and –OH at 3396cm^{-1} and C=O stretching of amide at 1674cm^{-1}. The ^{1}H-NMR and IR data confirms the vinyl phenol impurity **6** and hence it confirms as 2-{1-[2-(4-Hydroxy-3-vinyl-phenyl)-ethyl]-pyrrolidin-3-yl}-2,2-diphenyl acetamide (vinyl phenol, **6**).

CONCLUSION

Information about the different possible impurities, and their synthetic procedures were a prerequisite for a thorough understanding of the impurity formation pathway of the M_3 muscarinic receptor antagonist darifenacin hydro bromide. Keeping in view of the regulatory importance of darifenacin impurities, the process-related impurities and degradents in bulk darifenacin hydro bromide were identified, synthesized and characterized using mass, LC-MS, IR, and NMR techniques.

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Table-1: Physical and Analytical Data of Impurities 2,3,4,5 and 6.

Compd	MR (°C)	Molecular formula	Analysis % Calcd/Found		
			C	H	N
2	233-237	$C_{28}H_{30}N_2O_2$	78.84/79.01	7.09/7.22	6.57/6.79
3	242-244	$C_{28}H_{29}NO_3$	78.66/7.81	6.84/7.02	3.28/3.52
4	210-213	$C_{27}H_{29}NO$	84.55/84.63	7.62/7.80	3.65/3.75
5	199-201	$C_{32}H_{40}N_2O_3$	76.77/76.53	8.05/8.25	5.60/5.43
6	215-218	$C_{28}H_{30}N_2O_2$	78.84/78.71	7.09/7.11	6.57/6.70

Table-2: Spectral Data of Impurities 2,3,4,5 and 6

Compound	Mass (m/z)	IR KBr (cm^{-1})	^{1}H NMR (δ-ppm)
2	426	3466 (-N-H amide), 3094 (aromatic –C-H), 2956, 2906 (aliphatic –C-H), 1668 (-C=O),	Pyrrolidine: 2.15 (m, 1H, 4Ha), 2.83 (m, 2H, 4He, 5Ha), 3.18 (t, 1H, 2Ha), 3.55 (t, 1H, 2He), 3.68 (m, 1H, 5He), 3.90 (m, 1H, 3H), 3.03 (m, 4H, N-CH_2-CH_2), 3.11(t, 2H, Ph-CH_2), 4.51 (t, 2H, O-CH_2), 5.5 (s, 1H, NH, D_2O exchangeable),

		1248 (-C-N)	6.2 (s, 1H, NH, D_2O exchangeable), 6.62 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 7.0 (s, 1H, Ar-H), 7.19-7.43 (m, 10H, Ar-H of two phenyl)
3	427	3466 (broad, -O-H acid), 3059 (aromatic –C-H), 2962, 2924 (aliphatic –C-H), 1602 (-C=O), 1245 (-C-N)	Pyrrolidine: 2.20 (m, 1H, 4Ha), 2.90 (m, 2H, 4He, 5Ha), 3.15 (t, 1H, 2Ha), 3.45 (t, 1H, 2He), 3.62 (m, 1H, 5He), 4.05 (m, 1H, 3H), 3.4 (m, 4H, N-CH_2-CH_2), 3.3 (t, 2H, Ph-CH_2), 4.55 (t, 2H, O-CH_2), 6.65 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 6.95 (s, 1H, Ar-H), 7.10-7.55 (m, 10H, Ar-H of two phenyl).
4	383	3025 (aromatic –C-H), 2970, 2928 (aliphatic –C-H), 1244 (-C-N)	Pyrrolidine: 1.50 (m, 1H, 4Ha), 1.91 (m, 2H, 4He, 5Ha), 2.25 (t, 1H, 2Ha), 2.65 (m, 1H, 2He), 3.20 (m, 1H, 5He), 2.55 (m, 1H of 3H), 3.18 (m, 4H, N-CH_2-CH_2), 2.8 (t, 2H, Ph-CH_2), 3.75 (d, 1H of benzhydrol), 4.58 (t, 2H, O-CH_2), 6.71 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 7.0 (s, 1H, Ar-H), 7.10-7.35 (m, 10H, Ar-H of two phenyl)
5	500	3470 (-N-H amide), 3378 (-O-H acid), 3059 (aromatic –C-H), 2964, 2926 (aliphatic –C-H), 1674 (-C=O), 1265 (-C-N), 1217,1078 (-C-O)	Pyrrolidine: 2.11 (m, 1H, 4Ha), 2.73 (m, 2H, 4He, 5Ha), 3.15 (t, 1H, 2Ha), 3.35 (t, 1H, 2He), 3.52 (m, 1H, 5He), 3.81 (m, 1H, 3H), 0.98 (t, 3H, CH_3), 1.15 (d, 3H, CH_3), 1.45(m, 2H, CH_2), 3.03 (m, 4H, N-CH_2-CH_2), 3.11(t, 2H, Ar-CH_2), 3.5 (s, 1H, -O-H of phenol), 4.1 (m, 1H, -O-CH), 4.70 (t, 2H, O-CH_2), 5.5 (br, 2H, NH_2, D_2O exchangeable), 6.67 (d, 1H, Ar-H), 6.82 (d, 1H, Ar-H), 6.95 (s, 1H, Ar-H), 7.21-7.43 (m, 10H, Ar-H of two phenyl).
6	426	3396 (-N-H amide), 3055 (aromatic –C-H), 2987, 2906 (aliphatic –C-H), 1674 (-C=O), 1265 (-C-N)	Pyrrolidine: 2.06 (m, 1H, 4Ha), 2.82 (m, 2H, 4He, 5Ha), 3.22 (t, 1H, 2Ha), 3.62 (t, 1H, 2He), 3.78 (m, 1H, 5He), 3.90 (m, 1H, 3H), 3.15 (m, 4H, N-CH_2-CH_2), 5.28 (d, 1H, CH_2 of olephinic), 5.6 (br, 2H, NH_2, D_2O exchangeable), 5.70 (d, 1H, CH_2 of olephinic), 6.6 (d, 1H, Ar-H), 6.75 (d, 1H, Ar-H), 6.90 (dd, 1H, CH_2 of olephinic), 7.20 (s, 1H, Ar-H), 7.29-7.55 (m, 10H, Ar-H of two phenyl)

- ^{1}H-NMR Spectra of 2 to 6 were recorded in $CDCl_3$ and DMSO-d_6 at 400 MHz.

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