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Note

Synthesis/Isolation of darifenacin hydrobromide by-products

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During the process development in the laboratory, the purity of darifenacin hydrobromide has been tested by HPLC. Three by-product peaks along with darifenacin **1** peak have been observed whose area percentage ranged from 0.06-0.20% by HPLC. As per the stringent regulatory requirements, the by-products above threshold of ≥0.1% must be identified and characterized. All thorough study has been undertaken to synthesize and characterize these identified by-products. All the synthesized/isolated compounds have been co-injected with darifenacin hydrobromide, the relative retention times (RRT) are found to exactly match with the identified by-products. Based on their analytical and spectral data (HPLC, IR, NMR and mass), these by-products have been characterized as (3*S*)-*N*, *N*-bis[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]-2,2-diphenyl-2-(pyrrolidin-3-yl)acetamide (dimer-1), (3*S*)-*N*-[2-(2,3-dihydrobenzofuran-5-yl)-ethyl]-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)-ethyl]-pyrrolidin-3-yl}-2,2-diphenyl-acetamide (dimer-2) and (3*R*)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)-ethyl]pyrrolidin-3-yl}-2,2-diphenylacetamide (*R*-isomer).

Keywords: Darifenacin HBr, dimer-1, dimer-2, *R*-isomer, NMR data

Darifenacin (**1**, Enablex®) is a selective M3 muscarinic receptor antagonist for the treatment of overactive bladder[1-7]. Overactive bladder is a chronic and debilitating condition, caused by the untimely contraction of the bladder muscle and resulting in urinary urgency. Blockade of destructor muscle activity manifests in an increase in urine volume that the bladder can contain, reduction of urination frequency, and decrease in pressure and urgency associated with the urge to urinate, and thereby episodes of incontinence are reduced[8-10].

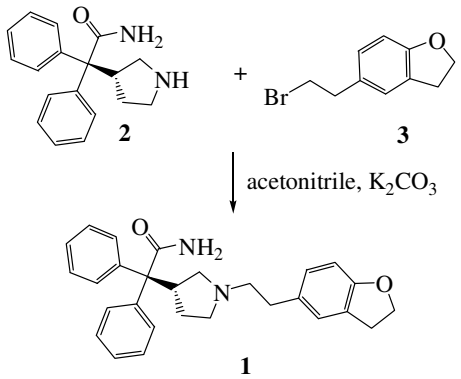
The literature survey revealed synthetic method for darifenacin hydrobromide[1,8-10]. It was synthesized by reacting 3-(*S*)-(-)-(1-carbamoyl-1,1-diphenylmethyl)-pyrrolidine **2** with 5-(2-bromoethyl)benzo[2,3-*b*]furan **3** in presence of anhydrous K_2CO_3 in acetonitrile to get 3-(*S*)-(-)-(1-carbamoyl-1,1-diphenylmethyl)-1-[2-(benzofuran-5-yl)ethyl]pyrrolidine **1** followed by column chromatographic purification using a mixture of dichloromethane and methanol (**Scheme I**)[1].

A novel method has been developed with slight modifications to make it simpler and commercially viable. During the process development of darifenacin hydrobromide, three by-products: dimer-1, dimer-2 and *R*-isomer are identified ranging from 0.06-0.20% by HPLC. Literature survey revealed that isolation and characterization of oxidized darifenacin, desnitrile impurity, ether impurity and vinyl phenol impurity are reported[10,11] and no information is available on identified by-products. Since it is impossible to eliminate by-products in a drug substance, a number of guidance documents have been drafted to prepare the known by-products present in the lab batches of darifenacin hydrobromide[12,13]. In this context, a comprehensive study was undertaken to isolate and characterize all the three by-products by spectroscopic and spectrometric techniques. The pathway for the formation of by-products and synthesis / isolation of by-products is also discussed in this paper.

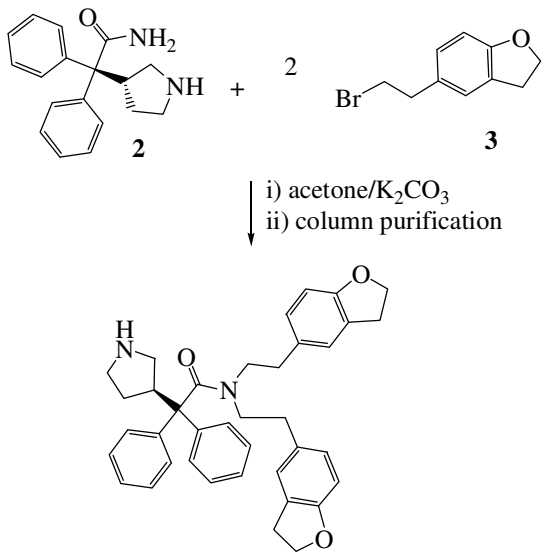
Results and Discussion

Detection of by-products dimer-1, dimer-2 and *R*-isomer

A typical analytical LC chromatogram of laboratory batch of darifenacin hydrobromide was recorded using the LC method and by-products peaks identified at RRT 1.28, 1.43 and 0.81. The same



Scheme I — Synthesis of darifenacin **1**



dimer-1

Scheme II — Synthesis of dimer-1

samples have been analyzed by LCMS method and mass number for the corresponding peaks are at *m/z* 572 (dimer-1), 572 (dimer-2), and 426 (*R*-isomer). The by-products were synthesized /isolated in the laboratory for structure elucidation and were co-injected with darifenacin hydrobromide, the RRT exactly matching with by-products.

Structure elucidation of dimer-1

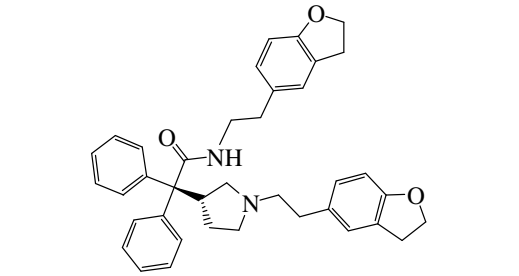
The dimer-1 is formed during the condensation of amide derivative **2** with bromo compound **3**. The compound was isolated from the column chromatography of syrup obtained from mother liquors of darifenacin. The mass spectrum showed the protonated molecular ion peak at *m/z* 573 atomic mass units which is 145 atomic mass units more than darifenacin. The IR spectra (KBr, cm^{-1}) of dimer-1 showed peaks at 3220 for amine function (sharp) and 1698 for carbonyl function. ^1H and ^{13}C NMR spectra indicate the presence of two moieties of 5-ethylene-2,3-dihydro-1-benzofuran in the dimer-1. The D_2O exchange experiment confirms the presence of only one exchangeable proton at δ 8.15 and it shows the correlation with pyrrolidine protons confirmed by gradient correlation spectroscopy (gCOSY) experiment. The gCOSY experiment confirms the pyrrolidine spin system and concludes that the pyrrolidine –NH is free. The methylene groups (C-8, C-8') of 5-ethylene-2,3-dihydro-1-benzofuran moieties shows the correlation with one quaternary (C-9, C-9') carbon in gradient heteronuclear multiple quantum coherence (gHMBC) experiment. The ^1H and ^{13}C NMR chemical shift values for H-7 and H-7' are more deshielded than darifenacin hydrobromide which confirms that the two 5-ethylene-2,3-dihydro-1-benzofuran moieties are attached to nitrogen atom of amide group. Based on the above spectral information, the molecular formula of the compound was found to be $C_{38}H_{40}N_2O_3$ and is unambiguously characterized as *N*,*N*-bis[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]-2,2-diphenyl-2-(pyrrolidin-3-yl)acetamide. Further, authentic sample has been prepared by condensing the amide derivative **2** with 2 moles of bromo compound **3** in acetone containing K_2CO_3 (**Scheme II**).

Structure elucidation of dimer-2

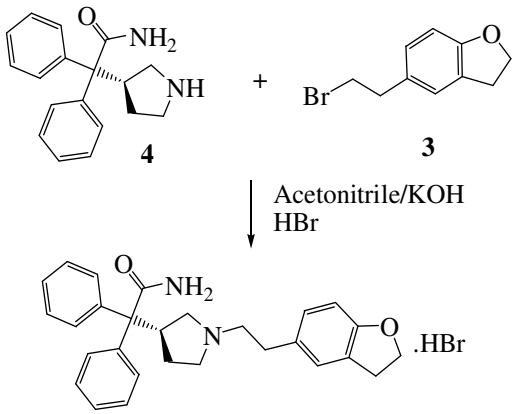
The dimer-2 is formed during the condensation of amide derivative **2** with bromo compound **3**. The compound is isolated from the column chromatography of syrup obtained from mother liquors of darifenacin. The mass spectrum showed the protonated molecular ion peak at *m/z* 572 atomic mass units similar to dimer-1. The IR spectra (KBr, cm^{-1}) of dimer-2 showed peaks at 3419 (broad) and 1654 indicating the presence of amide bond. The ^1H and ^{13}C NMR spectral data indicates the presence of one 5-ethylene-2,3-dihydro-1-benzofuran moiety on darifenacin and the chemical shift values of two dimers are not overlapping. The D_2O exchange experiment shows the presence of only one exchangeable proton at δ 5.62, which shows the correlation with ethylene protons (23-H) by gCOSY experiment. The two different methylene groups (C-8, C-24) of 5-ethylene-2,3-dihydro-1-benzofuran moiety shows the correlation with two different quaternary carbons (C-9, C-25) in gHMBC experiment and confirms that the two moieties are attached on different nitrogen atoms. These spectral evidences confirms the dimer-2 molecular formula as $C_{38}H_{40}N_2O_3$ and is unambiguously characterized as *N*-[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]-2-{1-[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl] pyrrolidin -3-yl}-2,2-diphenylacetamide.

Structure elucidation of *R*-isomer

The *R*-isomer is formed during the condensation of amide derivative **2** with bromo compound **3** (**Scheme III**). Further, authentic sample has been prepared by condensing the 3-(*R*)-(1-carbamoyl-1,1-



Structure of dimer-2



Scheme III — Synthesis of *R*-isomer

diphenylmethyl)pyrrolidine **4** with bromo compound **3** in acetonitrile containing KOH followed by saltification with 48% HBr. The mass spectrum showed the protonated molecular ion peak at *m/z* 427 which is equal to the mass of darifenacin hydrobromide. The IR spectrum (KBr, cm^{-1}) showed peaks at 3467 and 3211 for amine function and 1668 for carbonyl function and is similar to darifenacin hydrobromide. The ^1H and ^{13}C NMR spectrum patterns and chemical shift values are similar to that of darifenacin hydrobromide. Based on the above spectral information, the molecular formula of the compound was found to be $C_{28}H_{30}N_2O_2$.HBr and unambiguously characterized as 2-{(3*R*)-1-[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]pyrrolidin-3-yl}-2,2-diphenylacetamide hydrobromide.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Spectrum FT-IR spectrometer by using 1% KBr pellet method. All NMR experiments were performed on a Bruker AVANCE-300 instrument with a 5-m BBO probe head equipped with shielded Z-gradient coil at 298 K using solutions of 5 mg (for ^1H NMR) and 30 mg (for ^{13}C NMR) of the compound dissolved in 0.6 mL of $CDCl_3$ / DMSO-d_6. The data were collected and processed by XWIN-NMR software (Bruker) running on a PC with Microsoft Windows-XP. For the ^1H NMR analysis, 16 transients were acquired with a 1-s-relaxation delay using 32 K data points. The 90°pulse duration was of 11 μs and spectral width 6.000 kHz. The ^{13}C NMR and DEPT experiments were carried out with a spectral width of 16.500 kHz using 64 K data points. The two-dimensional experiments were performed using Bruker standard pulse sequences and parameters. The ^1H–^1H bond correlations were confirmed by gCOSY experiment (cosygpqf). The protonated carbon positions were confirmed by a gHSQC experiment (hsqcetgpsi2). The nonprotonated carbons were confirmed by a gHMBC experiment (hmbcgplpndqf). The ^1H chemical shifts are reported in ppm with reference to tetramethylsilane (δ 0.0). The ^{13}C chemical shifts were referenced to the central peak of the solvent molecule $CDCl_3$ (δ 77.00) or DMSO-d_6 (δ 39.50). All mass spectra are recorded on Agilent 1100 Series LC-MSD-TRAP-SL system. The electrospray ion source operated in positive mode with a needle voltage of 1500 V and a cone voltage of 4500 V in the scan mass range 150–650 (*m/z*). Nitrogen was used as nebulizer and curtain gas. Mass spectra were obtained using a Agilent 1100 Series LC-MSD-TRAP-SL system. The samples were introduced *via* the Direct Inlet Probe (DIP). The percentages of Carbon, Hydrogen and Nitrogen were obtained by using Thermo Finnigan elemental analyzer with thermal conductivity detector.

Spectral data of darifenacin hydrobromide: ^1H NMR (300 MHz, $CDCl_3$): δ 3.88-3.92 (m, 1H), 2.13-2.17 (m, 1H), 2.81-3.28 (m, 1H), 2.81-3.28 (m, 1H), 3.46-3.54 (m, 1H), 2.81-3.28 (m, 2H), 2.81-3.28 (m, 1H), 3.70 (m, 1H), 2.81-3.28(m, 2H), 6.86 (d, *J*=8.1 Hz, 1H), 6.65, (d, *J*=8.1 Hz, 1H), 7.02 (s, 1H), 2.81-3.28, (m, 2H), 4.53, (t, *J*=8.7 Hz, 2H), 7.20-7.37 (m, 4H), 7.20-7.37 (m, 4H), 7.20-7.37 (m, 2H), 5.58-5.78 (brs, 2H, NH_2), 11.44 (brs, 1H, N^+H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.79, 29.41, 30.98, 43.38, 55.73, 56.14, 56.81, 62.60, 71.09, 109.19, 125.10, 127.50, 127.53, 127.66, 127.92, 128.52, 128.64, 128.85, 128.90, 141.90, 142.09, 159.07, 175.13.

(3*S*)-2-[N-[2-(2,3-Dihydrobenzofuran-5-yl)ethyl]-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl] pyrrolidin-3-yl}]-2,2-diphenylacetamide (dimer-1): 2,2-Diphenyl-2-pyrrolidin-3-yl-acetamide (**2**, 2.8 g, 0.01 mol)

and 5-(2-bromoethyl)-2, 3-dihydrobenzofuran (**3**, 4.54 g, 0.02 mol) were refluxed in acetone (70 mL) containing K_2CO_3 (2.76 g, 0.02 mol). After completion of the reaction, the reaction mass was concentrated under vacuum and the obtained residue was poured into water. The separated solid was filtered and dried. The crude product was purified over silica gel column using MDC: MeOH (99:1 to 96:4) as eluent to obtain the product in 4 g yield. IR (KBr): 3220, 3056, 2946, 2856, 1698, 1614,1491, 1443,1359, 1243,1219, 1101,1076, 814,752,701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (m, 1H), 1.40-1.44 (m, 1H), 2.41-2.61 (m, 2H), 2.41-2.61 (m, 2H), 2.75-3.16 (m, 1H), 2.78 (m, 1H), 3.08 (t, *J*=8.6 Hz, 2H), 3.20-3.25 (m, 1H), 3.23-3.60 (m, 1H), 3.38-3.43 (m, 1H), 4.44 (t, *J*=8.9 Hz, 2H), 6.62 (d, *J*=8.1 Hz, 1H), 6.84 (d, *J*=8.1 Hz, 1H), 6.90 (d, *J*=6.9 Hz, 4H), 6.98 (s, 1H), 7.20-7.33 (m, 2H), 7.40 (d, *J*=6.6 Hz, 4H), 8.15 (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.55, 29.06, 32.22, 40.06, 43.41, 50.94, 59.25, 70.58, 108.36, 125.16, 126.33, 126.51, 126.96, 127.46, 127.69, 127.77, 128.55, 132.13, 141.18, 141.73, 157.81, 176.85; DIP MS: *m/z* (%) 573 $[M+H]^+$(100). Anal. Calcd for $C_{38}H_{40}N_2O_3$: C, 79.69; H, 7.04; N, 4.89. Found: C, 79.48; H, 7.10; N, 4.79%.

(3*S*)-2-{1-[2,4-Bis-(2,3-dihydrobenzofuran-5-yl)butyl]pyrrolidin-3-yl}-2,2-diphenylacetamide (dimer-2): Upon work-up of darifenacin, pharma mother liquors were collected and concentrated. The resultant residue was passed through a silica gel column using MDC: MeOH (96:4) to isolate dimer-2. IR (KBr): 3419, 3054, 2923, 1654, 1492, 1444, 1361, 1243, 1105, 816,752,703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.86-1.92 (m, 2H), 2.62, (t, *J*=6.5 Hz, 2H), 2.75-3.16 (m, 2H), 2.75-3.16 (m, 2H), 2.75-3.16 (m, 2H), 2.75-3.16 (m, 2H), 2.75-3.16 (m, 2H), 2.82-3.42 (m, 1H), 3.23-3.60(m, 2H), 3.70-3.74 (m, 1H), 3.75-3.80 (m, 1H), 4.53 (t, *J*=8.7 Hz, 2H), 4.54 (t, *J*=8.7 Hz, 2H), 5.62 (brs, 1H), 6.60-6.67 (m, 1H), 6.60-6.67 (m, 4H), 6.60-6.67(m, 1H), 6.79 (s, 1H), 6.87 (d, *J*=8.1 Hz, 1H), 7.02-7.08 (m, 1H), 7.02-7.08 (m, 2H), 7.25-7.29 (m, 4H), 7.25-7.29 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.23, 29.58, 29.67, 31.36, 34.77, 40.92, 44.04, 53.87, 56.33, 57.26, 62.99, 71.17, 71.24, 109.29, 109.36, 125.05, 125.29, 127.36, 127.41, 127.65, 127.86, 128.07, 128.07, 128.63, 128.92, 129.03, 129.79, 142.65, 158.91, 159.19,172.74; DIP MS: *m/z* (%) 573 $[M+H]^+$(100). Anal. Calcd for $C_{38}H_{40}N_2O_3$: C, 79.61; H, 7.02; N, 4.71. Found: C, 79.48; H, 7.10; N, 4.79%.

(3*R*)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-pyrrolidin-3-yl}-2,2-diphenylacetamide (*R*-isomer) hydrobromide: The bromo compound **3** (4.54 g, 0.02 mol) and 3-(*R*)-(1-carbamoyl-1,1-diphenylmethyl)-pyrrolidine (**4,** 5.6 g, 0.02 mol) were refluxed in acetonitrile containing KOH (1.12 g, 0.02 mol). The reaction mixture was distilled off, water (50 mL) and MDC (50 mL) was added to the obtained residue to form a two-phase mixture. The phases were separated and organic phase was distilled under vacuum. The obtained residue was dissolved in methyl ethyl ketone (10 mL) and filtered to eliminate un-dissolved solid. The 48% HBr (0.344 g, 0.00204 mol) was added to the filtrate and distilled under vacuum to afford solid foam. The foam was scurried in diisopropylether and filtered to get the title compound in 7 g yield. IR (KBr): 3468, 3260, 3211 3098, 2960, 2906, 2856, 2696, 2602, 1668, 1584, 1493, 1479,1442, 1350, 1248,1217, 1105,1083, 814, 774, 765, 705 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.01-2.19 (m, 1H), 2.41-2.61(m, 2H), 2.82-3.42 (m, 1H), 2.82-3.42 (m, 1H), 2.82-3.42 (m, 2H), 2.82-3.42 (m, 2H), 2.82-3.42 (m, 2H), 3.45-3.53 (m, 1H), 3.74-3.96 (m, 1H), 4.54 (t, *J*=8.7 Hz, 2H), 5.59-5.71 (brs, 2H, NH_2), 6.66, (d, *J*=7.8 Hz, 1H), 6.87 (d, *J*=7.5 Hz, 1H), 7.02 (s, 1H), 7.20-7.44 (m, 2H), 7.20-7.44 (m, 4H), 7.20-7.44 (m, 4H), 11.53 (brs, 1H, N^+H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.02, 29.55, 31.15, 43.66, 56.28, 56.98, 62.71, 71.22, 109.36, 125.25, 127.64, 127.68, 128.05, 128.19, 128.59, 128.78, 128.94, 129.07, 142.06, 142.38, 159.22, 175.25; DIP MS: *m/z* (%) 427 $(M+1-HBr)^+$. Anal. Calcd for $C_{28}H_{31}BrN_2O_2$: C, 66.27; H, 6.16; N, 5.52; Br, 15.75. Found: C, 66.31; H, 6.09; N, 5.48; Br, 15.98%.

Conclusion

In conclusion, the present investigation provides isolation and characterization of dimer-1, dimer-2 and *R*-isomer. The present investigation also provides simple synthetic methods to prepare dimer-1 and *R*-isomer in multi-gram scale. These products are very useful for toxicological studies, validation studies and have regulatory importance. It is hoped that the present efforts to synthesize and characterize them effectively will prove to be valuable.

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References

1 Cross P E & Mackenzie A R D, *EP* 0388054, **1990**; *Chem Abstr*, 114, **1990**, 247125.
2 Barry K, William J H, Paul V M, Sylvia R, David A S, Richard F V & William W, *Anal Chem*, 68, **1996**, 1658.
3 Robert M W & Carolyn M N, *Life Sciences*, 64, **1999**, 395.
4 Yamada S, Maruyama S, Takagi Y, Uchida S & Oki T, *Life Sciences*, 80, **2006**, 127.
5 Smith C M &Wallis R M, *Journal of Receptor and Signal Transduction Research*, 17, **1997**, 177.
6 Choppin A & Eglen R M, *Brit J Pharmacol*, 133, **2001**, 1035.
7 Beaumont K C, Cussans N J, Nichols D J & Smith D A, *Xenobiotica*, 28, **1998**, 63.
8 Merli V, Canavesi A, Daverio P & Vailati A, *PCT Int Appl* WO 2007076157, **2003**; *Chem Abstr*, 147, **2007**, 143270.
9 Merli V, Canavesi A & Daverio P, *PCT Int Appl* WO 2007076158, **2003**; *Chem Abstr*, 147, **2007**, 143269.
10 Merli V, Canavesi A & Daverio P, *PCT Int Appl* WO 2007076159, **2003**; *Chem Abstr*, 147, **2007**, 118125.
11 Srinivas K, Rajeshwar R S, Mahesh R G, Dubey P K, Chakravarthy A K & Pratap R P, *Rasa J Chem*, 2, **2009**, 151.
12 *ICH Guideline* Q3A (R), February, **2002**.
13 *ICH Guideline* Q3B (R), February, **2002**.