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Synthesis and characterization of new related substances of the antiarrhythmic drug dronedarone hydrochloride

Marina Santos[a], Lia C. García[b], Cintia Checura[a], Lucía Gandolfi Donadío[b], Carlos Fernandez[c], Hernán Orgueira[c], Maria J. Comin[b,*]

[a] Laboratory of Analytical Research and Process Control, Center of Research and Development in Chemistry, National Institute of Industrial Technology, Buenos Aires, Argentina
[b] Laboratory of Organic Synthesis, Center of Research and Development in Chemistry, National Institute of Industrial Technology, Buenos Aires, Argentina
[c] MAPRIMED S.A., Av Directorio 6155, C1440ATA-CABA, Argentina

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ABSTRACT

Two new potential impurities of antiarrhythmic drug substance Dronedarone Hydrochloride together with debutyldronedarone were detected by LC–MS analysis during process development. A successful synthetic strategy for the synthesis of these potential impurities was developed facilitating the access to new impurity reference standards. Their synthesis and characterization are discussed in detail. The availability of these impurity standards allowed cost reduction through the increase of process control.

1. Introduction

Dronedarone hydrochloride (**1**), marketed as Multaq® by Sanofi-Aventis [1], was approved by the US FDA in 2009 for the treatment of nonpermanent atrial fibrillation and atrial flutter [2]. Dronedarone was developed with the intent of replicating the effects of the antiarrhythmic drug Amiodarone (**2**), while minimizing its significant toxicity. Like Amiodarone, Dronedarone is a benzofuran derivative, but with different relative electrophysiological activities on individual ion channels. Specific structural modifications were introduced in order to minimize the non-cardiovascular adverse effects of Amiodarone such as accumulation in adipose tissue and thyroid dysfunction [3]. Thus, a methanesulfonamide group was added to shorten half-life and decrease lipophilicity, and the iodine atoms were eliminated to avoid the risk of thyroid side effects (Fig. 1) [4].

The control of impurities in the manufacture of active pharmaceutical ingredients (API) is critical in delivering an API of high quality. Furthermore, the safety of a drug product is dependent not only on the toxicological properties of the active drug substance itself, but also on the impurities that it may contain. Therefore, identification, quantification, and control of impurities in the drug substance and drug product, are an important part of drug development and regulatory assessment [5].

The nature and the quantity of impurities in the drug products are typically governed by different factors such as the synthetic route and the reaction conditions, the quality of the starting materials, reagents and solvents, the purification steps, the excipients, the drug product manufacturing processes, the packaging and storage of the end product. The level of total impurities permitted is typically less than 1.0%, and those for each individual impurity at levels of 0.05–0.1% (depending on the maximum daily dose). At this level, the individual impurities must be identified and quantified. This results in a constant demand for impurity reference standards for both regulatory authorities and pharmaceutical companies. Therefore, it is important to develop synthetic methods in addition to detection methods for these compounds, once they have been identified.

Most of the synthetic processes for preparing Dronedarone Hydrochloride use the cost-contributing intermediate (**3**) as starting material [6–8]. Briefly, phenol **3** is alkylated with 1-chloro-3-di-n butylaminopropane hydrochloride (**4**) and the resulting intermediate **5** is then reduced by hydrogenation. The obtained

* Corresponding author at: Av Gral Paz 5445, B1650WAB, San Martin, Buenos Aires, Argentina. Fax: +54 11 4724 6289.
E-mail address: jcomin@inti.gob.ar (M.J. Comin).

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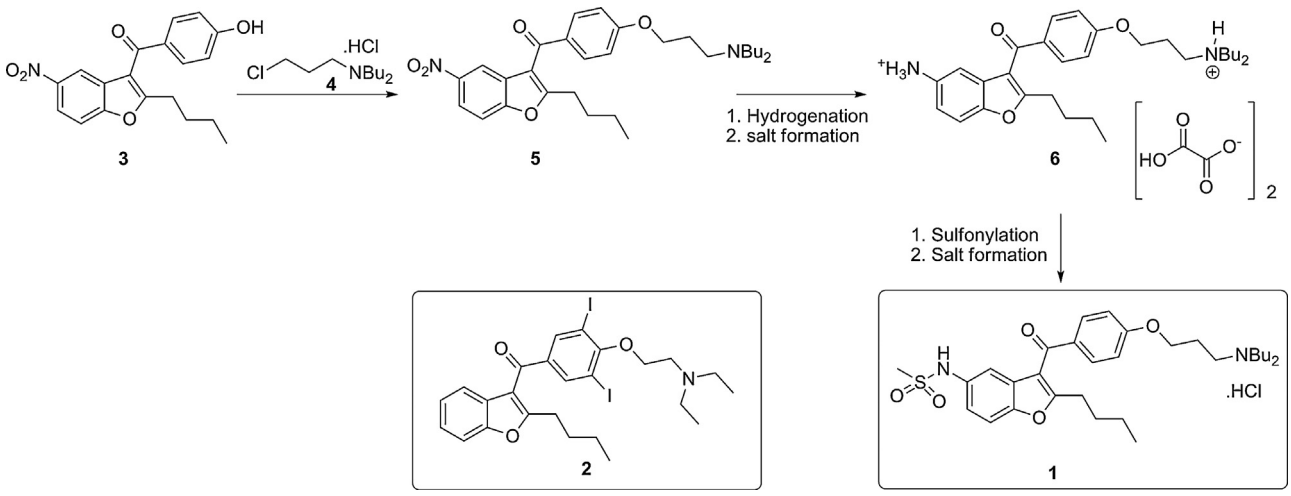


Fig. 1. Synthesis of Dronedarone Hydrochloride (**1**). Structure of Amiodarone (**2**).

aniline derivative, isolated as dioxalate salt **6**, is treated with methanesulfonyl chloride to yield Dronedarone free base that is subsequently isolated as the hydrochloride salt **1** (Fig. 1).

The structures of known Dronedarone related substances are shown in Fig. 2. They include the synthetic intermediates **3**, **5** and **6** and the disulfonamide derivative known as impurity B (**7**) [9], that is a by product from the sulfonylation step. In addition, debutyldronedarone (**8**), which is known as the major circulating metabolite after oral administration of Dronedarone, is also included here [10,11]. A recently published study has identified, synthesized and characterized several impurities that were previously unknown [12]. In this report, Mahender and coworkers described: process related impurities V (**9**) and VI (**10**) that present modifications on the sulfonamide moiety, impurity IV (**11**), that originates from the presence of impurities with a shorter hydrocarbon chain in the benzofuran starting material, as well as a new chloromethane sulfonyl derivative (impurity III, **12**) that results from the use of impure chlorosulfonylmethane reagent.

During our API process development efforts of Dronedarone Hydrochloride (**1**), various process-related substances have been identified. Among the main impurities, the already known related substances **6**, **7**, **8** and **11** were detected during LC–MS analysis. As shown in the results section, two previously unknown impurities postulated as structures **13** and **14** could also be detected during the same analysis (Fig. 2). Upon examination of the synthetic process employed for the manufacture of Dronedarone, we postulate compounds **8**, **13** and **14** as potential process related impurities of the API derived from the presence of debutylated impurities in the alkylating agent (**4**) used. Indeed, *N*-(3-Chloropropyl) butan-1-amine and 3-chloropropan-1-amine are common contaminants of *N*-butyl-*N*-(3-chloropropyl) butan-1-amine (**4**). Purification process to completely eliminate them results in cost increasing. However, the identification and control of the resulting impurities formed downstream the process, like **8**, **13** and **14**, allowed us to maintain some level of impurities at the key starting material (**4**) and at the same time, produce Dronedarone within specifications at a lower cost. To the best of our knowledge, compounds **13** and **14** have not been reported in the literature to date. Compound **8** is known as the main Dronedarone active metabolite, debutyldronedarone [11], but its synthesis has not been reported. In this paper we present the synthesis and characterization of these three compounds together with the LC–MS analysis of several laboratory and validation batches where these substances have been detected.

2. Experimental

2.1. General Procedures

All chemical reagents were commercially available and were obtained from Sigma–Aldrich (Argentine) unless otherwise noted. Nitrobenzofurane starting material **3** (99.5%) was purchased from Zhejiang Sanmen Kangning Chemical Co., Ltd., (China). Column chromatography was performed on a Teledyne Isco CombiFlash Companion (USA) instrument under gradient elution conditions with RediSep disposable flash columns. Analytical TLC was performed on Merck silica gel 254F plates. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 (Germany) instrument at 400 and 100 MHz, respectively. Spectra are referenced to the solvent in which they were run (7.26 ppm for $CDCl_3$). Low resolution positive ion electrospray ionization (ESI) mass spectra were performed on a Waters Quattro Premier XE spectrometer (Waters, Milford, MA, USA). Low resolution positive ion electron impact ionization (EI at 70 eV) mass spectra were recorded on a Shimadzu QP 2010 Ultra spectrometer. FT-IR spectra were recorded using a Thermo Nicolet iZ10 FT-IR spectrophotometer elemental analyses were performed by UMYMFOR-CONICET, Argentine.

2.2. Liquid chromatography–mass spectrometry

The LC–ESI/MS and MS analysis were performed on a Waters Quattro Premier XE spectrometer (Waters, Milford, MA, USA) equipped with a Waters 2695 binary pump plus auto sampler. A XTerra MS C18 column (Waters, Milford, MA, USA, 100 mm × 2.1 mm, 3,5 μm) was used for chromatographic separation. Mobile phase A consisted of 0.1% (v/v) formic acid and mobile phase B consisted of 0,1% formic acid (v/v) in acetonitrile in gradient mode (T_{min} A:B) T_0 50:50, $T_{3.5}$ 20:80, T_6 20:80, T_7 50:50, T_{11} 50:50 with a flow rate of 0.4 mL/min was used. The injection volume was 10 μl. The column was maintained at 40 °C throughout the analysis.

The mass instrument was operated in electrospray positive ion mode, the source voltage was kept at 3,2 kV, the cone voltage at 40 V and the source temperature at 150 °C. Nitrogen was used for desolvation as well as the cone gas.

2.3. Supplementary information

The supplementary information relating to this work contains detail experimental procedures for the synthesis of new

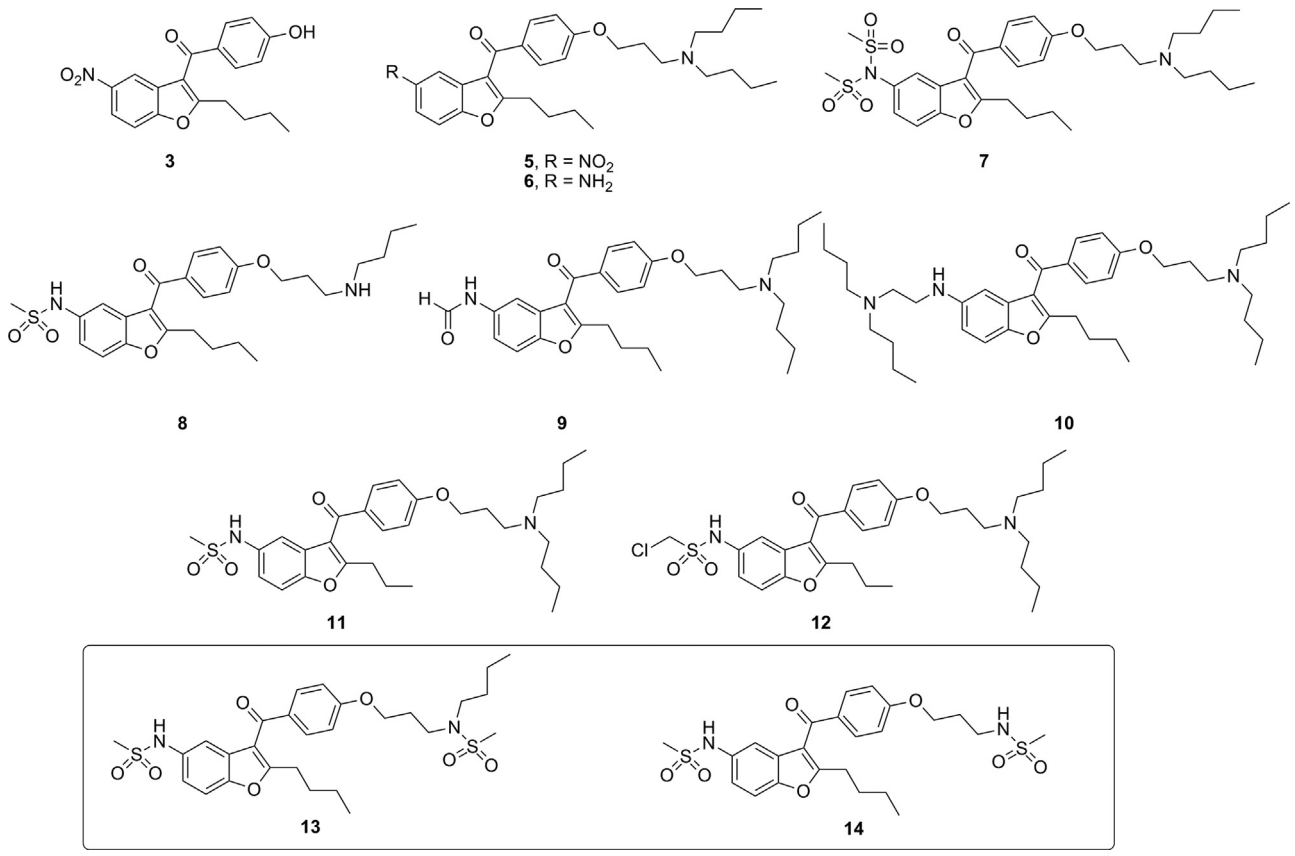


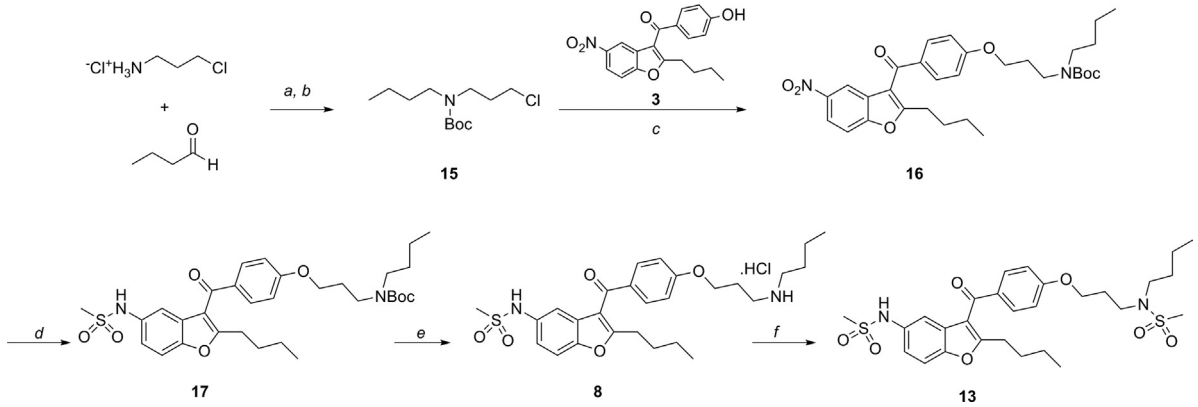
Fig. 2. Structures of Dronedarone related substances.

compounds, full characterization, ^{1}H, ^{13}C NMR, IR, EI Mass spectra and elemental analysis.

3. Results and discussion

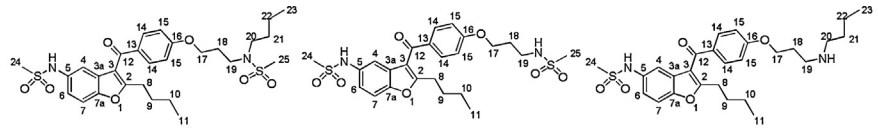
The synthetic strategy employed for the preparation of monobutylated derivatives **8** and **13** is depicted in Scheme 1. Tert-Butyloxycarbonyl (Boc)-protected *N*-(3-chloropropyl) butan-1-amine (**15**), that was prepared from butyraldehyde and 3-chloropropylamine by reductive amination followed by protection of the secondary amine, was used as alkylating agent. Thus, reaction of phenol **3** with **15** under standard bimolecular nucleophilic substitution (SN_2) conditions conducted to nitro derivative **16** in 67% yield. Subsequently, **16** was treated with hydrogen gas (H_2(g)) in the presence of platinum–copper (Pt–Cu) catalyst. The crude product thus obtained was subjected to sulfonylation conditions to afford the monosulfonamide product **17** in 68% yield after two synthetic steps and purification by column chromatography (CC). Finally, the Boc protecting group was removed yielding the desired related substance **8** in quantitative yield. The disulfonamide derivative **13** was prepared by reacting **8** with one equivalent of methanesulfonyl chloride (MsCl) in 82% yield with 99.7 area% purity (Scheme 1).

The structure of the new compound **13** was confirmed by its ^{1}H and ^{13}C NMR spectra (Table 1): the signals corresponding to the



Scheme 1. Synthesis of process related monobutylated substances **8** and **13**. Reagents and Conditions: (a) i. MeOH, rt; ii. $NaBH_3CN$; (b) Boc_2O, CH_2Cl_2, 0 °C, 93% (two steps) ; (c) K_2CO_3, KI, anhydrous DMF, 60 °C, 67% ; (d) i. MeOH, H_2; (g) (45 psi), Pt–Cu/C, rt; ii. MsCl, THF, TEA, 0 °C, 68% (two steps); (e) 4 M HCl–IPA, 0 °C → rt; (f) MsCl, THF, TEA, 0 °C, 82% (two steps).

Table 1
Numbering and NMR assignment of Dronedarone process related substances **13**, **14** and **8**.



13 **14** **8**

	Impurity **13**			Impurity **14**			Impurity **8**		
C[a]	Number of protons (H)	δ ^{1}H (ppm), multiplicity, *J* (Hz)	δ ^{13}C (ppm)	Number of protons (H)	δ ^{1}H (ppm), multiplicity, *J* (Hz)	δ ^{13}C (ppm)	Number of protons (H)	δ ^{1}H (ppm), multiplicity, *J* (Hz)	δ ^{13}C (ppm)
2	-	-	166.4	-	-	165.1	-	-	166.3
3	-	-	116.7	-	-	116.9	-	-	116.7
3a	-	-	128.2	-	-	127.8	-	-	128.1
4	1H	7.21, *d*, *J* = 2.0	115.5	1H	7.27, *s*	112.1	1H	7.19, *s*	115.2
5	-	-	151.8	-		150.8	-	-	151.7
6	1H	7.28, *dd*, *J* = 8.7; 2.0	120.0	1H	7.20, *d*, *J* = 8.8	119.3	1H	7.45, *d*, *J* = 8.7	119.7
7	1H	7.43, *d*, *J* = 8.7	111.9	1H	7.62, *d*, *J* = 8.8	113.6	1H	7.30, *d*, *J* = 8.7	111.8
7a	-	-	131.7	-	-	134.8	-	-	131.4
8	2H	2.88, *m*	28.0	2H	2.80, *t*, *J* = 7.4	27.7	2H	2.85, *m*	27.9
9	2H	1.74, *m*	30.1	2H	1.65, *m*	29.9	2H	1.74, *m*	29.7
10	2H	1.38-1.28, *m*	20.0*	2H	1.24, *m*	22.1	2H	1.37-1.25, *m*	20.4*
11	3H	0.93, *t*, *J* = 7.4**	13.8**	3H	0.81, *t*, *J* = 7.3	13.9	3H	0.92-0.88, *m*	13.7**
24	3H	2.90, *s*	39.0	3H	2.90, *s*	39.7	3H	2.91, s	39.0
12	-	-	190.3	-	-	189.7	-	-	190.1
13	-	-	132.4	-	-	131.4	-	-	132.6
14	1H	7.79, *d*, *J* = 8.8	131.6	1H	7.78, *d*, *J* = 8.4	131.9	1H	7.79, *d*, *J* = 8.7	131.6
15	1H	6.95, *d*, *J* = 8.8	114.4	1H	7.08, *d*, *J* = 8.4	114.9	1H	6.94, *d*, *J* = 8.7	114.3
16	-	-	162.7	-	-	163.0	-	-	162.9
17	2H	4.14, *t*, *J* = 6.1	65.2	2H	4.14, *t*, *J* = 6.0	65.8	2H	4.14, *t*, *J* = 6.1	66.2
18	2H	2.13, *m*	28.7	2H	1.95, *m*	29.6	2H	2.03, *m*	28.6
19	2H	3.39, *t*, *J* = 6.9	45.0	2H	3.14, *m*	40.0	2H	2.85, *m*	46.2
20	2H	3.20, *t*, *J* = 7.7	48.3	-	-	-	2H	2.65, *m*	49.3
21	2H	1.60, *m*	30.7	-	-	-	2H	1.52, *m*	31.9
22	2H	1.38-1.28, *m*	22.4*	-	-	-	2H	1.37-1.25, *m*	22.3*
23	3H	0.88, *t*, *J* = 7.3**	13.7**	-	-	-	3H	0.92-0.88, *m*	13.7**
25	3H	2.86, *s*	37.9	3H	2.88, *s*	39.1	-	-	-

[a] Refer the structural formulas for numbering
*,** Signals could be interchanged;

sulfonamide methyl substituents appeared as singlets centered at *δ* 2.90 and *δ* 2.86 ppm in the ^{1}H NMR and again as two different signals observed at *δ* 39.0 and *δ* 37.9 ppm in the ^{13}C NMR. In contrast, in case of the known related substance impurity B (**7**), only one singlet that integrates for 6 protons was observed centered at *δ* 3.38 ppm according to the presence of two equivalent methylsulfonamide moieties. The ESI–MS showed a protonated molecular ion peak at *m*/*z* 579 confirming the molecular weight of **13** as 578 (Fig. 3C). The IR spectrum displayed characteristic absortions at 3168 cm^{-1} (sulfonamide NH), 1626 cm^{-1} (C=O), 1328 and 1144 cm^{-1} (SO_2) and 912 cm^{-1} (S—N). The structure of *N*-debutyldronedarone (**8**) was also similarly confirmed. Its ^{1}H NMR showed a singlet at *δ* 2.91 ppm that integrates for 3 protons and corresponds to the methylsulfonamide group and the ESI–MS showed a protonated molecular ion at

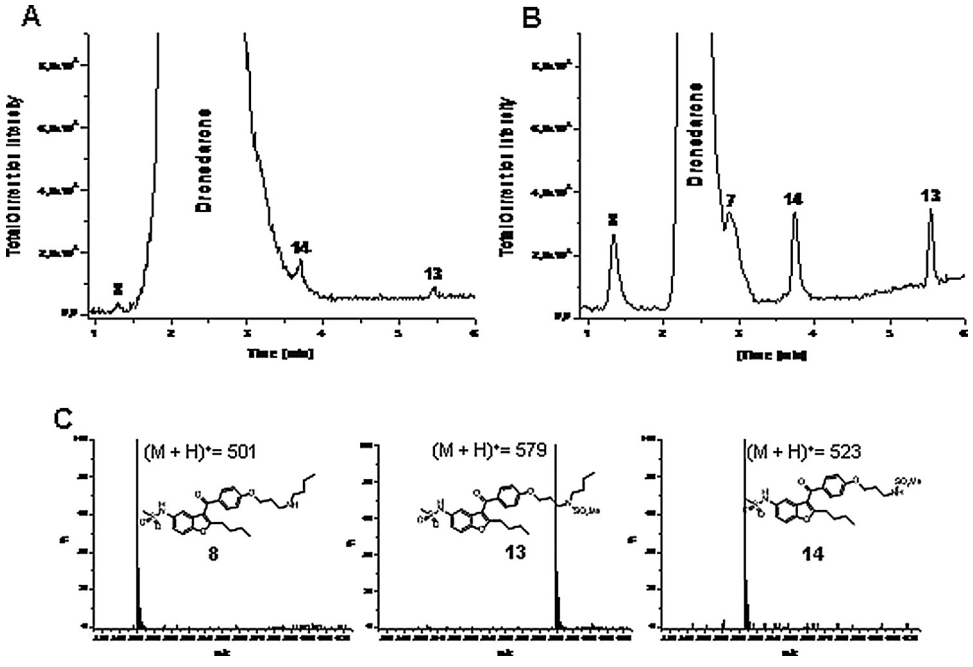
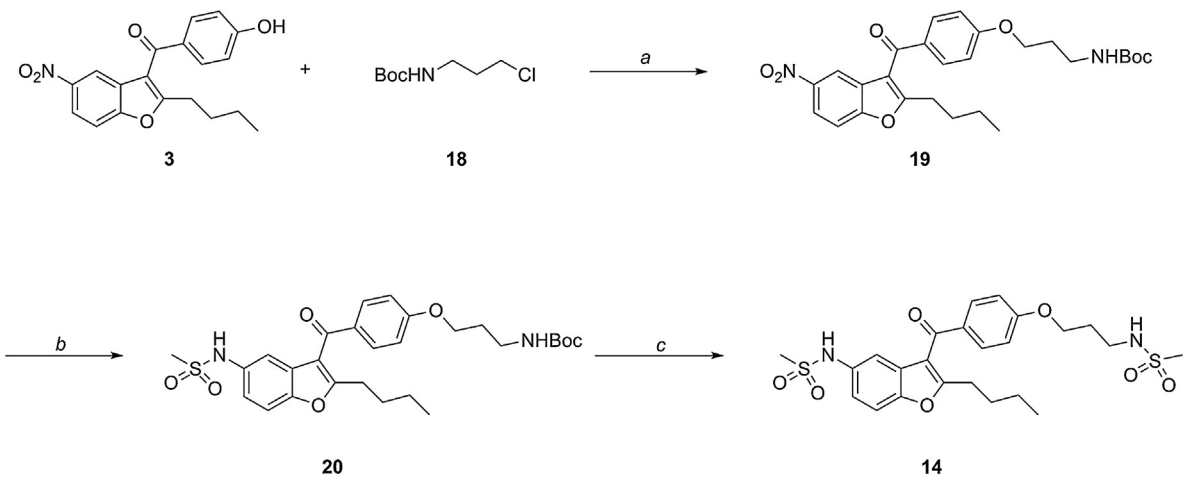


Fig. 3. (**A**) LC–MS chromatogram of a representative validation batch of Dronedarone (250 μg/mL); (**B**) LC–MS chromatogram of a representative validation batch of Dronedarone (50 μg/mL) sample spiked with synthesized impurities at the 0.1% level. The target impurities are marked as **8** (retention time (RT): 1.3 min), **14** (RT: 3.8 min), and **13** (RT: 5.5 min). The peak marked as **7** (RT: 2.9 min) is the known related substance, impurity B; (**C**) ESI–MS of LC peaks corresponding to each new impurity.



Scheme 2. Synthesis of process related debutylated substance **14**. Reagents and Conditions: (a) K_2CO_3, KI, anhydrous DMF, 60 °C, 90% ; (b) i. MeOH, H_2(g) (45 psi), Pt–Cu/C, rt, ii. MsCl, THF, TEA, 0 °C, 60% (two steps); (c) i. 4 M HCl–IPA, 0 °C → rt; ii. MsCl, THF, TEA, 0 °C, 67% (two steps).

m/*z* 501 in accordance with the molecular weight of the proposed structure (500) (Fig. 3C). The IR spectrum displayed characteristic absortions at 3189 cm^{-1} (sulfonamide NH), 1624 cm^{-1} (C=O), 1327 and 1148 cm^{-1} (SO_2), and 907 cm^{-1} (S—N). Finally, the results obtained in the elemental analysis fully support the molecular formula postulated for compounds **13** and **8**.

Debutylated process related substance **14** was prepared in a similar fashion starting from phenol **3** and *N*-Boc-protected 3-chloropropylamine **18**. In this case, the alkylation reaction gave product **19** in 90% yield after column chromatography purification. Subsequent reduction of the nitro group followed by sulfonylation of the resulting aniline derivative afforded intermediate **20** in 60% overall yield. Then, removal of Boc protecting group followed by treatment of the resulting intermediate with one equivalent of MsCl and triethylamine (TEA) at 0 °C, conducted to the desired compound **14** in 67% yield with 98.5 area% purity after two recrystallizations from isopropyl alcohol (IPA) (Scheme 2). The positive ESI–MS spectrum of compound **14** exhibited a protonated molecular ion peak at *m*/*z* 523, consistent with the molecular mass of the proposed structure (Fig. 3C). Moreover, ^1H and ^{13}C NMR spectra are in full agreement with the debutylated structure (Table 1). In this sense, as in the case of impurity **13**, two non equivalent methylsulfonamide moieties could be proposed from the observed ^1H and ^{13}C NMR diagnostic signals. Accordingly, two singlets centered at δ 2.90 and δ 2.88 ppm and integrating for three protons each, were seen in the ^1H NMR and two different signals were observed at δ 39.7 and δ 37.1 ppm in the ^{13}C NMR, respectively. The IR spectrum displayed characteristic absortions at 3262 cm^{-1} (sulfonamide NH), 1624 cm^{-1} (C=O), 1328 and 1140 cm^{-1} (SO_2), and 913 cm^{-1} (S—N). Lastly, the results obtained in the elemental analysis fully support the molecular formula postulated for compound **14**.

The analysis of Dronedarone and its impurities was performed as described in the experimental section. This reversed-phase LC–MS method can adequately separate the components in less than 6 min. Several samples of Dronedarone from different batches were analyzed using the above method. The results of these analyses showed small quantities (below quantitation level) of the three target impurities **8**, **13** and **14**. Fig. 3A and B show representative LC–MS chromatograms of a Dronedarone sample alone and one spiked with the impurities at the 0.1% level, respectively.

4. Conclusions

New potential process related substances of the API dronedarone have been synthesized and thoroughly characterized by MS, NMR and IR. LC–MS analysis of several validation batches showed the presence of **8**, **13** and **14** at low levels. Their presence can be attributed to the reaction of the starting phenol **3** with *N*-(3-chloropropyl) butylamine and *N*-(3-chloropropyl) amine and subsequent hydrogenation and sulfonylation steps. Access to these impurities standards enabled their process control, and made possible to raise the limit for its precursors upstream the process within the key starting material (**4**), in order to keep the Dronedarone impurity profile under specification and at the same time optimize cost reduction.

In summary, we have confirmed the identity of three potential related substances of Dronedarone Hydrochloride. Compounds **8**, **13** and **14** were successfully synthesized in gram scale in 45, 36, 37% yields, respectively. Additionally, a LC method that allowed their quantification in less than 6 min was developed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2015.06.026

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