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Effect of deuteration on the metabolism and clearance of some pharmacologically active compounds—synthesis and *in vitro* metabolism of deuterated derivatives of dronedadrone†

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The synthesis and *in vitro* metabolism studies of a family of specifically deuterated derivatives of dronedarone are described. Metabolic stability and clearance of the parent compound are not sensitive to deuterium substitution, irrespective of the position of the heavy label.

Keywords: stable labelled synthesis; deuterium; deuterium labelling; isotope effect; antiarrhythmic; *in vitro* metabolism

Introduction

Recent years have seen a renaissance of interest in the development of deuterated drug molecules. Up to the year 2000, patent applications concerning deuterated pharmaceuticals remained at a low but fairly constant level, but by the end of the first decade of the 21st century, activity in this area had increased dramatically.[1] For example, in 2002, only two applications for deuterated drugs were recorded, a number which had risen to 64 by 2010. Over the same time period, although the number of new drug entities approved for the market annually remained stable, overall pharmaceutical R&D spending approximately doubled.[2] Clearly, a significant portion of the R&D effort in this area is driven by economic considerations: substitution of hydrogen for deuterium in late-stage or marketed molecules creates a patentable, usually active chemical entity without the expense incurred from lengthy discovery programs to identify hits and transform them to lead molecules. Based on this economic model, several start-up companies have made this activity their core business.[3]

The scientific rationale behind attempts to improve absorption, distribution, metabolism and excretion properties of drug candidates by deuteration has been comprehensively reviewed[4–6] and is based on the primary isotope effect: C-D bonds are stronger than the corresponding C-H bonds, so that any oxidative metabolism in which C-H bond-breaking is at least partially rate-limiting, could theoretically be slowed down if hydrogen is replaced by deuterium at the metabolic 'hot-spot'. The *in vivo* half-life of, and exposure to the parent molecule should thus be increased, transforming drug candidates, which had previously failed because of the lack of exposure, into promising candidates once again. Although such efforts have been underway since the 1970s, no deuterated entities have yet made it to market, the most promising results to date concern MK-0641 that was withdrawn in clinical phase II,[7] and SD-809 reported to be currently approaching phase III[8] (Figure 1).

The theoretical considerations outlined above have proved difficult to translate to *in vivo* success. In an effort to understand which structural parameters are important to enhance metabolic stability of deuterated compounds compared to their unlabelled parents, we have prepared a series of deuterated analogues of dronedarone, a compound from our company portfolio and studied their metabolic profiles *in vitro* compared to the unlabelled parent.

Dronedarone, marketed since 2009 as Multaq ®, is a treatment for atrial fibrillation and is a substituted benzofuran for which the

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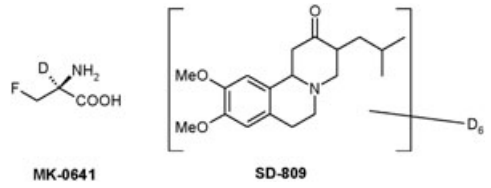
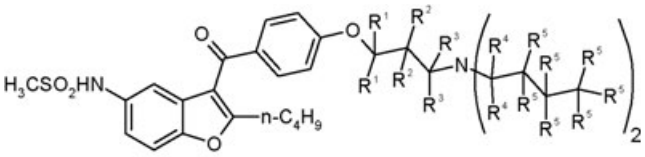


Figure 1. Chemical Structures of MK-0641 and SD-809.

metabolic behaviour in man is well established. The major, active metabolite, SR35021, results from cytochrome P450 (CYP) mediated debutylation, with the isoform 3A4 of the enzyme being the major contributor.[9] SR35021 is further metabolised to the inactive carboxylic acid SR95014 by monoamine oxidase-mediated oxidative deamination.[10] Other minor metabolic pathways involve *O*-dealkylation to phenol **1** and some oxidation/hydroxylation to a series of uncharacterised derivatives **2**.[10] (Scheme 1). In order to investigate the sensitivity of the major oxidative pathways to deuterium substitution and its effect on metabolic clearance of dronedarone, we prepared a series of eight specifically deuterated analogues **3a-h** with D atoms in α positions to the principal sites of metabolism **3a**, **3e**, **3g** and **3h** or with per-deuterated chains **3c**, **3b**, **3f** or with combinations of both substitution types **3d**. The compounds containing per-deuterated chains, that is, **3c**, **3b**, **3f** and **3d** were prepared to include any minor chain hydroxylation pathways in the investigation. These compounds were incubated separately with human hepatocytes in primary culture under standard conditions, and the incubates analysed quantitatively for dronedarone over 24 h.

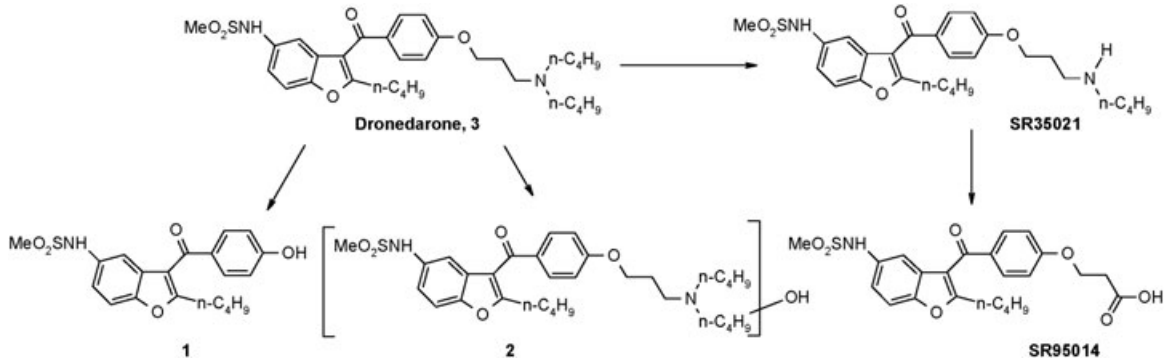


3a R^1=D and R^2, R^3, R^4, R^5 = H
3b R^1, R^2, R^3, R^4, R^5 = D
3c R^1, R^2, R^3 = D and R^4, R^5 = H
3d R^1, R^2, R^3, R^4 = D and R^5 = H
3e R^3 = D and R^1, R^2, R^4, R^5 = H
3f R^4, R^5 = D and R^1, R^2, R^3 = H
3g R^3, R^4 = D and R^1, R^2, R^5 = H
3h R^4 = D and R^1, R^2, R^3, R^5 = H

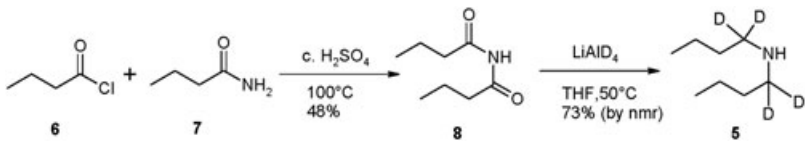
Results and discussions

Chemistry

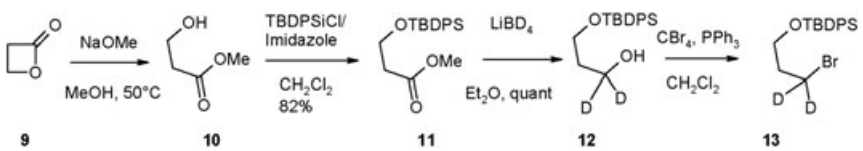
For the deuterated dronedarone derivatives completely labelled in the butyl side chains, **3b** and **3f**, the key per-deuterated di-n-



Scheme 1. Human metabolic pathways for dronedarone, **3**.



Scheme 2. Synthesis of deuterated dibutylamine, **5**.



Scheme 3. Synthesis of deuterated propyl derivative **13**.

buylamine is commercially available, whereas for the compounds α-deuterated to nitrogen in the side chains, that is, **3d**, **3g** and **3h**, it was necessary to prepare the D4 amine **5** (Scheme 2). Butyryl chloride, **6**, and butanamide, **7**, were condensed under acidic conditions, using a modification of a literature procedure[11] to yield the imide, **8**, which was reduced to **5** in good yield using lithium aluminium deuteride.

Similarly, for the targets per-deuterated in the central propyl chain, **3b** and **3d**, D6-bromopropanol is readily available. For analogues carrying D atoms α to the phenolic O atom, **3a-d**, we prepared methyl-3-hydroxypropionate, **10**, using β-propiolactone, **9**, according to a literature procedure.[12] *O*-Protection to **11**,[12] followed by deuteroreduction of the ester function, gave a very good yield of the mono-protected deuterated diol, **12**, which was brominated under standard conditions to compound **13** (Scheme 3).

β-propiolactone, **9**, was also used in the synthesis of analogues **3e** and **3g** carrying D atoms α to the tertiary nitrogen atom. Starting from the readily available nitro-substituted phenol, **14**, base mediated opening[13] gave the propionic acid, **15**, which was deutero-reduced to the intermediate alcohol, **16**, prior to activation as the tosyl derivative **17**. Reaction of **17** with the previously described deuterated amine, **5** or D9-n-butylamine, gave the two nitro derivatives **18e** and **18g** respectively. (Scheme 4)

The remaining nitro derivatives **18a-18d**, **18f** and **18h** were all prepared from **14** using the intermediates described above. Thus, **14** was converted to a series of ethers **19a-c** by reaction with either D6-dibromopropane, dibromopropane or **13** under a variety of similar conditions. **19c** was deprotected and brominated under standard conditions to bromo derivative **19d**. Alkylation of the three amines **9**, d9-di-n-butylamine and di-n-butylamine with the appropriate bromo derivatives **19a**, **b** or **d** gave the nitro derivatives **18a-18d**, **18f** and **18h** (Scheme 5).

All eight nitro compounds **18a-h** were converted to their methylsulphhonylamido derivatives **3a-h** by tin(II) chloride reduction to the primary amines **20a-h**, following a literature procedure.[14] The primary amines **20a-h** were then sulphonylated using excess methanesulphonyl chloride in the presence of aqueous ammonia. This technique is used to ensure that any *bis* sulphonylated material, **21**, is hydrolysed immediately to the required **3**. All analogues were isolated as their hydrochloride salts (Scheme 6).

In vitro metabolism studies

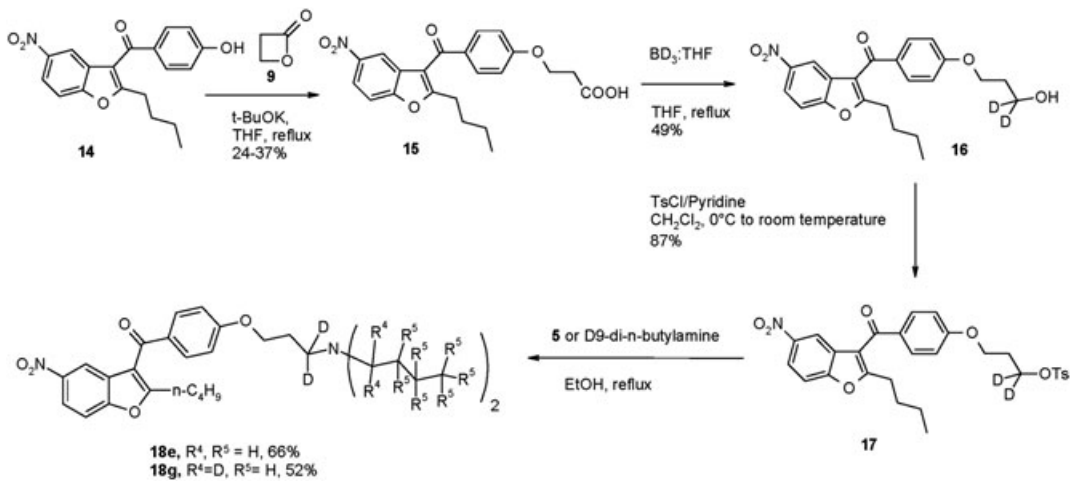
With the deuterated analogues **3a-h** in hand, each was incubated separately with two different preparations of human hepatocytes, as was unlabelled dronaderone. The experiments were carried out at two different substrate concentrations (0.5 and 5 μM) either alone, in presence of ketaconazole, **22**, or in presence of 1-aminobezotriazole, **23**. (Figure 2). These latter incubations were designed to quantify the contributions of both CYP3A4, and total CYPs to the overall metabolic clearance of the analogues, because **22** selectively inhibits the **3A4** isoform[15], whereas **23** inhibits the whole family of p450 enzymes.[16] Each incubate was analysed by liquid chromatography-mass spectrometry (LC-MS) over time to quantify the concentration of starting dronedarone analogue remaining.

Figure 3 shows a typical time-concentration plot for dronedarone, **3**, and its eight deuterated analogues, **3a-h**, over a 24 h incubation with one specific human hepatocyte preparation.

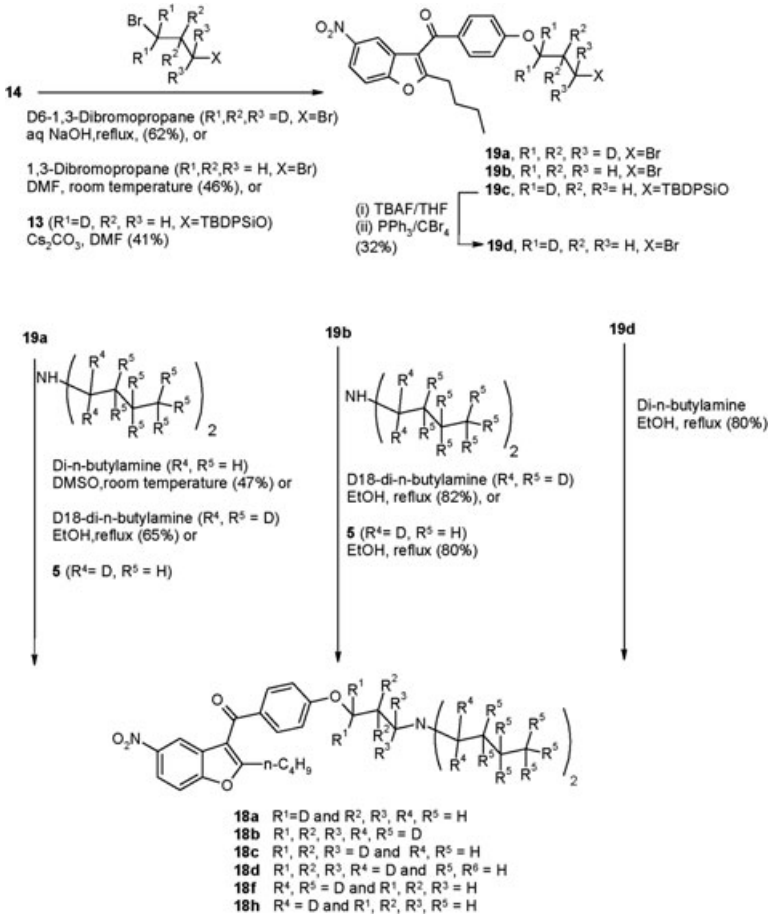
That no significant differences exist in the metabolic stability of any of the analogues, is supported by the calculated mean clearance figures for the whole set of incubations (Figure 4), obtained at two different substrate concentrations (0.5 and 5 μM) with two different human hepatocyte preparations. We assume that no saturation of clearance occurs, and that there is no difference in CYP contribution over the concentration range. Additionally, from the incubations conducted in presence of **22** and **23**, no significant differences in either the contribution of CYP3A4 or total CYP to the overall metabolic clearance could be discerned (Figure 4).

Experimental

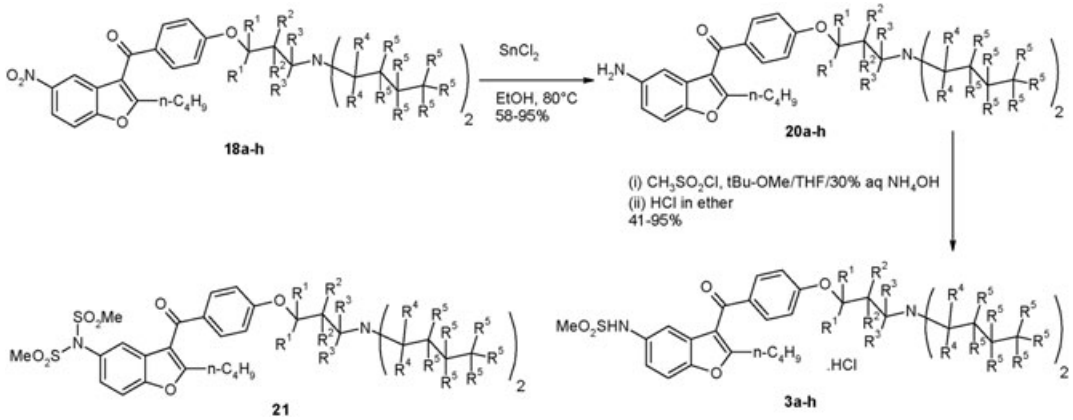
All reagents were of commercial quality and were used as received. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel containing fluorescence indicator (silica gel 60 F254, from Merck KGaA) or by LC/MS (Agilent 1100 Series; column: Symmetry C18, 5 μm, 4.6 × 50 mm; mass detector: API3000 from AB-SCIEX). Purifications by column chromatography were carried out on cartridges of pre-packed silica gel 60, (0.063–0.2 mm) from Merck Chimie or Macherey-Nagel with the described eluents. The ^{1}H NMR and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) or on a Bruker Avance 600 (600 MHz) nuclear magnetic resonance spectrometer, ^{13}C-NMR spectra being obtained using off-resonance decoupling so that



Scheme 4. Synthesis of nitro derivatives α deuterated in propyl chain **18e**, **18g**.



Scheme 5. Synthesis of nitro derivatives **18a-d**, **18f** and **18h**.



Scheme 6. Conversion of nitro compounds, **18** to methylsulphonamido derivatives, **3**.

carbon atoms carrying deuterium atoms appeared as multiplets. The ^{1}H NMR data were compared with literature data or checked against an authentic sample of the corresponding unlabelled compound. Where analytical data is not recorded for a particular analogue, characterisation was achieved by comparison of chromatographic data with a more fully characterised analogue. The MS spectra were recorded on a Shimadzu ion trap/time-of-flight mass spectrometer.

N-Butyrylbutyramide (8)

A mixture of butyryl chloride, **6** (0.87 g, 10 mmol), butanamide, **7** (1.04 mL, 10 mmol), and several drops of concentrated H_2SO_4 was heated on an oil bath at 100°C for 2 h. The reaction mixture was partitioned between dichloromethane and saturated sodium carbonate solution, and the layers separated. The aqueous phase was extracted three times with dichloromethane, the combined organic phase dried over $MgSO_4$ and evaporated to an off white solid.

The condensation was repeated using **6** (8.70 g, 100 mmol) and **7** (10.4 mL, 100 mmol) and the crude products combined and recrystallised from diisopropyl ether to provide **8** (8.30 g, 53 mmol, 48%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.37 (bs, 1H), 2.55 (*t*, 4H), 1.80-1.60 (*m*, 4H), 1.00 (*t*, 6H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 174, 39, 18, 14; gas chromatography (GC) MS retention time = 13.42 min, m/z = 158 ($M+H^+$)

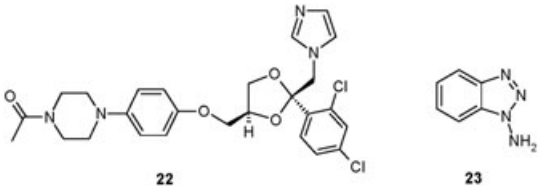


Figure 2. Chemical structures of ketoconazole, **22**, and 1-aminobenzotriazole, **23**.

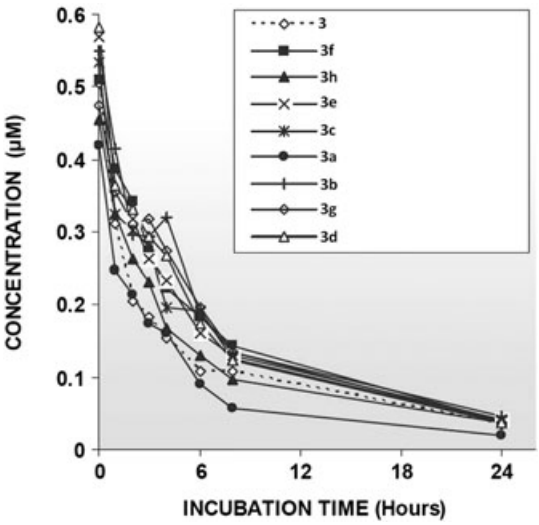


Figure 3. Time versus concentration plot during hepatocyte incubation for dronedarone and its deuterated analogues **3a-h**.

[α, α, α', α'-$^2H_4$]-dibutylamine (5)

A solution of **8** (3.00 g, 19 mmol) in anhydrous THF (40 mL) was treated with $LiAlD_4$ (1.20 g, 29 mmol). After warming for several hours, GC analysis showed reaction to be incomplete, so further $LiAlD_4$ (1.00 g, 24 mmol) was added and warming continued for 4 h. The mixture was quenched by the addition of excess 1 M sodium hydroxide solution, and extracted three times with dichloromethane. The combined extracts were dried over solid potassium carbonate and carefully evaporated to dryness at room temperature to avoid loss of volatile product. **5** (2.53 g containing residual solvent) was recovered as a mobile, practically colourless liquid. The yield, as estimated by ^{1}H NMR was 73%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.6-1.2 (*m*, 8H), 0.9 (*t*, 6H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 49.1(*m*), 32.3, 20.6, 14.2. MS *m/z* 134 ($M+H^+$).

Methyl-3-hydroxypropionate (10)[11]

A solution of β-propiolactone, **9** (20.15 g, 0.28 mol), in MeOH (100 mL) was treated portionwise at room temperature with sodium methoxide (1.51 g, 28 mmol) with stirring. The mixture was warmed on an oil bath at 50°C for 4 h, and after cooling to room temperature, the MeOH was evaporated and the residue taken up in diethyl ether. Insoluble material was removed by filtration through a pad of silica gel and the volatiles removed under reduced pressure at room temperature to give **10** as a pale yellow oil (23.5 g, 0.23 mol, 82%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.85 (*q*, 2H), 3.68 (s, 3H), 2.60(*t*, 2H), 2.38 (*t*, 1H) used without additional purification.

Methyl -3-(*tert*-butyldiphenylsilanyloxy)propioniate (11)

A stirred solution of **10** (5.00 g, 48 mmol) in anhydrous dichloromethane (100 mL) was treated with imidazole (6.54 g, 96 mmol) at room temperature. *Tert*-butyldiphenylchlorosilane (15 mL, 58 mmol) was added dropwise and the reaction mixture allowed to stir for 24 h at room temperature. Crushed ice was added and the mixture partitioned between water and dichloromethane. The layers were separated and the aqueous phase re-extracted twice with dichloromethane. The combined organic phase was washed with water, dried over anhydrous sodium sulphate and evaporated to yield a practically colourless, thick oil.

The reaction was repeated using the same quantities of reagents, and the products combined to give the crude intermediate as a thick oil (45 g). This was chromatographed on silica gel in 98:2 n-pentane/diethyl ether to give **11** (27.0 g, 79 mmol, 82%) as a thick oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.75-7.65 (*m*, 4H), 7.5-7.35 (*m*, 6H), 3.90 (*t*, 2H), 3.70 (s, 3H) 2.60 (*t*, 2H), 1.06 (s, 9H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 172.2, 135.6, 133.5, 129.7, 127.7,127.6, 59.9, 51.6, 37.7, 26.7, 19.2.

[1-$^2H_2$]-3-(*tert*-Butyldiphenylsilanyloxy)propan-1-ol (12)[11]

A solution of **11** (5.00 g, 14.6 mmol) in diethyl ether (20 mL) was treated portionwise with $LiBD_4$ (188 mg, 7.3 mmol) over 20 min. GC analysis showed the reaction to be incomplete, so further $LiBD_4$ (311 mg, 12 mmol) was added, and the mixture allowed to stir overnight. The mixture was partitioned between water and diethyl ether, the layers separated and the aqueous phase extracted twice with diethyl ether. The combined ethereal phase was dried over $MgSO_4$, and evaporated to a colourless oil, which crystallised on standing as crude **12** (5.76 g containing some residual solvent), which was used in the next step

Compound	Mean Clearance in mL.h^{-1}.(10^6 hep)$^{-1}$	% CYP3A4 contribution	% Total CYP contribution
3	0.141 ± 0.041	86 ± 4	94 ± 4
3a	0.142 ± 0.042	88 ± 3	95 ± 2
3b	0.123 ± 0.044	84 ± 5	96 ± 2
3c	0.133 ± 0.044	84 ± 5	95 ± 2
3d	0.125 ± 0.039	84 ± 3	96 ± 4
3e	0.138 ± 0.048	85 ± 6	91 ± 5
3f	0.127 ± 0.049	84 ± 7	97 ± 5
3g	0.117 ± 0.032	81 ± 10	94 ± 6
3h	0.146 ± 0.048	89 ± 3	98 ± 2

Figure 4. Mean total in vitro metabolic clearances, CYP3A4 and total CYP contributions characterizing *in vitro* metabolism of dronedarone **3** and deuterated analogues **3a-h**.

without purification. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.75–7.35 (*m*, 10H), 3.97 (*t*, 2H), 2.60 (bs, 1H), 1.84 (bt, 2H), 1.14(*m*, 9H).

[3-$^2H_2$]-(3-Bromopropoxy)-*tert*-butyldiphenylsilane (13)[11]

A mixture of **12** (5.76 g containing solvent, 14.6 mmol based on starting **11**) and triphenylphosphine (4.54 g, 17.3 mmol) in dichloromethane (12 mL) was treated with a solution of carbon tetrabromide (6.69 g, 20.2 mmol) in dichloromethane (15 mL). After 30 min, the mixture was concentrated, taken up in n-pentane and the insoluble triphenylphosphine oxide removed by filtration. The solution was concentrated to give crude **13** (7.55 g), which was used without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.73–7.35 (*m*, 10H), 3.82 (*t*, 2H), 2.10 (*m*, 2H), 1.15(*m*, 9H)

3-[4-(2-Butyl-5-nitro-benzofuran-3-carbonyl)phenoxy]-propionic acid (15)

A mixture of **14** (1.00 g, 2.95 mmol), β-propiolactone, **9** (0.212 g, 2.95 mmol) and THF (10 mL) was stirred under argon at room temperature and treated dropwise with a 1 M solution of potassium *tert*-butoxide in THF (2.95 mL, 2.95 mmol). The reaction mixture was heated at reflux for 3 h, allowed to cool overnight, then treated with further **9** (0.109 g, 1.5 mmol) and 1 M potassium *tert*-butoxide in THF (1.5 mL, 1.5 mmol) and heated at reflux for 2.5 h. The mixture was cooled to room temperature, diluted with water and extracted twice with EtOAc. The combined extracts were dried over $MgSO_4$ and evaporated to a gum, which was chromatographed in 98:2 chloroform-MeOH to give **15** as a waxy solid (0.30 g, 0.72 mmol, 24%) ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.50 (bs, 1H), 8.28 (d, 1H), 8.20 (dd, 1H), 7.80 (d, 2H), 7.55 (d, 1H), 6.96 (d, 2H), 4.35 (*t*, 2H), 2.90 (2 x *t*, 4H), 1.80 (q^5, 2H), 1.30 (*m*, 2H), 0.87 (*t*, 3H).

The synthesis was repeated using **14** (2.00 g, 5.89 mmol), β-propiolactone, **9** (0.74 mL, 11.8 mmol) and solid potassium *t*-butoxide (1.321 g, 11.8 mmol), both added in two portions. Column chromatography under the same conditions gave further **15** (0.90 g, 2.19 mmol, 37%).

(2-Butyl-5-nitrobenzofuran-3-yl)-[4-(3-[$^2H_2$]-3-hydroxypropoxy)phenyl]-methanone (16)

15 (0.90 g, 2.19 mmol) in solution in THF (50 mL) stirred on an ice water bath under argon, was treated dropwise with a 1 M solution of borane-D3–THF complex in THF (2.4 mL, 2.4 mmol). After 3 h, further 1 M solution borane-D3–THF complex in THF (2.4 mL, 2.4 mmol) was added and stirring continued for 2.5 h. Saturated aqueous potassium carbonate solution was added, followed by ice water, and the mixture extracted twice with EtOAc. The combined extracts were washed with water, dried over $MgSO_4$ and evaporated to an oil.

The reduction was repeated twice using **15** (0.50 g, 1.22 mmol and 6.71 g, 13.3 mmol) and 1 M solution borane-D3–THF complex in THF (1.82 mL, 1.82 mmol and 31.5 mL, 31.5 mmol). Similar work-up and combination of reaction products gave crude **16** (7.38 g), which was chromatographed in chloroform: MeOH (995:5) on silica gel to yield purified **16** (3.30 g, 8.26 mmol, 49% overall) as a thick oil, which was used without further characterisation.

(2-Butyl-5-nitrobenzofuran-3-yl)-[4-(3-[$^2H_2$]-3-(4-methylphenylsulphonyloxy) propoxy) phenyl]-methanone (17)

16 (3.30 g, 8.26 mmol) dissolved in dichloromethane (100 mL) was treated with *p*-toluenesulphonyl chloride (3.15 g, 16.5 mmol), and the solution stirred on an ice water bath, while triethylamine (2.3 mL, 16.5 mmol) was added dropwise. After 20 h, further quantities of *p*-toluenesulphonyl chloride (3.15 g, 16.5 mmol) and triethylamine (2.3 mL) were added, and after an additional 24 h, more *p*-toluenesulphonyl chloride (1.57 g, 8.23 mmol) and triethylamine (1.15 mL, 8.3 mmol) were again added. The mixture was diluted with water, extracted twice with EtOAc and the combined extracts washed with water and dried over $MgSO_4$ before being evaporated. The residue was chromatographed on silica gel in 50:50 chloroform : n-pentane in a gradient elution up to 60:40 chloroform : n-pentane to provide **17** (4.00 g, 7.23 mmol, 87%). MS *m/z* 554 ($M+H^+$); 552 ($M-H^-$). The product was further characterised by comparison of its TLC properties with the unlabelled analogue.

(2-Butyl-5-nitrobenzofuran-3-yl)-[4-(3-dibutylamino-(3-[$^2H_2$]-propoxy)-phenyl]-methanone (18e)

A mixture of **17** (1.25 g, 2.26 mmol) and di-n-butylamine (0.96 mL, 5.6 mmol) in ethanol (30 mL) was heated at reflux for 8 h, with the addition of further di-n-butylamine (0.8 mL, 4.6 mmol) after 4.5 h. The reaction mixture was evaporated, and the residue chromatographed in 98:2 chloroform-MeOH on silica gel to yield, after evaporation **18e** (0.75 g, 66%), characterised by comparison of its TLC properties with the unlabelled analogue.

(2-Butyl-5-nitrobenzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylamino-(3-[$^2H_2$]-propoxy)-phenyl]-methanone (18g)

A mixture of **17** (1.25 g, 2.26 mmol) and **5** (0.752 g, 5.64 mmol) in ethanol (30 mL) was heated at reflux for 8 h, with the addition of further **5** (0.63 g, 4.4 mmol) after 4.5 h. The reaction mixture was evaporated, and the residue chromatographed in 98:2 chloroform-MeOH on silica gel to yield, after evaporation **18 g** (0.60 g, 52%), characterised by comparison of its TLC properties with the unlabelled analogue.

[4-(3-Bromo-[$^2H_6$]-propoxy)-phenyl]-(2-butyl-5-nitro-benzofuran-3-yl)-methanone (19a)

To a solution of **14** (3.243 g, 9.56 mmol,) in water (10 mL) was added [$^2H_6$]-1,3-dibromopropane (2.519 g, 12.1 mmol) and sodium hydroxide (0.352 g, 8.8 mmol). The reaction mixture was stirred at reflux for 8 h, allowed to warm to room temperature then diluted with water and dichloromethane. The aqueous layer was extracted three times with dichloromethane, and the combined organic layers were dried over Na_2SO_4, and evaporated. The brown oil so obtained was chromatographed in 9:1 pentane-diethyl ether on silica gel to give **19a** as a pale yellow oil (2.752 g, 62%).^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.3 (s, 1H), 8.1 (*d*, 1H), 7.8 (*m*, 2H), 7.5 (*d*, 1H), 7.0 (*d*, 2H), 2.9 (*t*, 2H), 1.75 (*q*, 2H), 1.3 (*q*, 2H), 0.9 (*t*, 3H). MS *m/z* 466 ($M+H^+$)

This experiment was repeated using **14** (4.00 g, 11.8 mmol), water (16 mL), [$^2H_6$]-1,3-dibromopropane (3.11 g, 15.0 mmol) and sodium hydroxide (0.434 g, 10.8 mmol) to provide a further quantity of **19a** as a pale yellow oil (3.10 g, 6.7 mmol, 56%) identical to the first batch.

[4-(3-Bromo-propoxy)-phenyl]-(2-butyl-5-nitro-benzofuran-3-yl)-methanone (19b)

To a solution of **14** (1.000 g, 2.94 mmol) in dimethylformamide (30 mL) was added 1,3-dibromopropane (0.373 mL, 3.65 mmol) under argon. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with water and EtOAc. The organic layer was separated, washed with water, dried over Na_2SO_4 and the solvent evaporated. The brown oil so obtained was chromatographed in 90:10 pentane-diethyl ether to give **19b** as a light yellow oil (0.625 g, 1.36 mmol, 46%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.4 (s, 1H), 8.2 (*d*, $J=8$ Hz, 1H), 7.8 (*d*, $J=8$ Hz, 2H), 7.6 (*d*, 1H), 4.2 (*t*, 2H), 3.6 (*t*, 2H), 2.9 (*t*, 2H), 2.4 (q^5, 2H), 1.8 (q^5, 2H), 1.3 (q^6, 2H), 0.9 (*t*, 3H). MS *m/z* 461 ($M+H^+$)

In an alternative reaction, **14** (1.323 g, 3.90 mmol), 1,3-dibromopropane (1.00 g, 4.95 mmol) and solid sodium hydroxide (0.15 g, 3.8 mmol) were heated together with stirring at reflux for 5 h. The mixture was diluted with water and extracted three times with dichloromethane. The combined extracts were dried over $MgSO_4$ and evaporated to a dark oil (2.1 g). This was chromatographed on silica gel in 90:10 n-pentane/diethyl

ether to give **19b** as a yellow oil (1.15 g, 2.50 mmol, 64%) identical to the previous batch.

{4-[3-(*tert*-Butyl-diphenyl-silanyloxy)-3-[$^2H_2$]propoxy]-phenyl}-(2-butyl-5-nitro-benzofuran-3-yl)-methanone (19c)

A mixture of crude **13** (7.55 g, 14.4 mmol based on starting **11**), **14** (4.89 g, 14.4 mmol) and caesium carbonate (4.69 g, 14.4 mmol) in DMF (80 mL) was heated on an oil bath at 110°C. The mixture was concentrated under reduced pressure and the black residue chromatographed on silica gel in 95:5 pentane/diethyl ether to give **19c** (3.81 g, 6.0 mmol, 41% over the three steps) as a waxy solid, which was used directly in the next step. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.39 (s, 1H), 8.21 (*d*, 1H), 7.85 (*m*, 2H), 7.75-7.30 (*m*, 11H), 6.96 (*d*, 2H), 3.90 (*t*, 2H), 2.94 (*t*, 2H), 2.07 (*t*, 2H), 1.78 (*m*, 2H), 1.35 (*m*, 2H), 1.05 (s, 9H), 0.90 (*t*, 3H).

[4-(3-[$^2H_2$]-3-Bromopropoxy)-phenyl]-(2-butyl-5-nitro-benzofuran-3-yl)-methanone (19d)

A solution of **19c** (3.61 g, 5.7 mmol) in anhydrous THF (100 mL) was stirred and cooled on an ice-water bath at 0°C. Tetrabutylammonium fluoride solution (6.8 mL of 1 M in THF, 6.8 mmol) was added, and the dark mixture stirred at room temperature overnight. The mixture was poured onto crushed ice and extracted three times with EtOAc. The combined extracts were dried over magnesium sulphate and evaporated to yield crude alcohol intermediate (4.0 g) as an orange oil, which was chromatographed on silica gel in 98:2 dichloromethane/MeOH to provide the intermediate alcohol (1.00 g, 2.5 mmol, 44%) as a pale yellow oil, which was transferred to a reaction flask with dichloromethane (20 mL). Triphenylphosphine (790 mg, 3.0 mmol) and carbon tetrabromide (1.16 g, 3.5 mmol) were added, and the reaction stirred at room temperature. TLC analysis indicated that the reaction was incomplete, so further triphenylphosphine (393 mg, 1.5 mmol) and carbon tetrabromide (0.581 g, 1.75 mmol) were added and the mixture stirred overnight. The volatile solvents were evaporated, and the residue chromatographed on silica gel in 9:1 n-pentane/diethyl ether to provide **19d** (0.82 g, 1.8 mmol, 32% from **19c**). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.34 (1H, *d*), 8.21 (1H, dd), 7.82 (2H, *d*), 7.58(1H, *d*), 7.01 (*d*, 2H), 4.23 (*m*, impurity), 3.64 (*t*, 2H), 2.92 (*t*, 2H), 2.39 (*t*, 2H), 1.76 (*m*, 2H), 1.45-1.34 (*m*, 4H), 0.90 (*t*, 3H).

(2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-dibutylamino-[$^2H_6$]-propoxy)-phenyl]-methanone (18c)

A mixture of K_2CO_3 (0.475 g, 3.44 mmol) and **14** (1.001 g, 2.95 mmol) in butan-2-one (5.4 mL) was treated with [$^2H_6$]-1,3-dibromopropane (1.238 g, 5.95 mmol) under argon. The reaction mixture was stirred at reflux for 4.5 h, then allowed to cool to room temperature, diluted with methyl-*tert*-butyl-ether (20 mL), filtered and the solvents evaporated to yield a yellow oil. The oil so obtained was diluted with DMSO (7.5 mL) and treated with dibutylamine (1.2 mL, 7.06 mmol) and the suspension stirred at room temperature for 18 h. The reaction mixture was then partitioned between water and EtOAc. The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated to yield a brown oil, which was chromatographed in 99:1 dichloromethane-MeOH containing 0.1% concentrated ammonium hydroxide to give **18c** (0.718 g, 5.9 mmol, 47%) as a light orange oil. MS *m/z* 515 ($M+H^+$).

Synthesis of other tertiary amines (18a, 18b, 18d, 18f and 18h) from bromo compounds (19a, 19b and 19d)

A solution of the bromo derivative **19** in ethanol (~10 volumes) was treated with the appropriate dibutylamine (~2.5 molar equivalents) and heated for 5 h at reflux temperature. Further dibutylamine was added and heating continued until conversion was complete as shown by TLC. The reaction mixture was cooled and evaporated to dryness before the crude **18** was chromatographed in 98:2 dichloromethane-MeOH containing 0.2% concentrated ammonia solution on silica gel. By this general method the following were obtained:

(2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-di-butylamino-1-[$^2H_2$]-propoxy)-phenyl]-methanone (**18a**) (0.72 g, 1.41 mmol, 80%) was isolated as a yellow oil from **19d** (0.82 g, 1.77 mmol) and di-n-butylamine (total of 1.2 mL, 7.0 mmol)

(2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-di-[$^2H_9$]-butylamino-[$^2H_6$]-propoxy)-phenyl]-methanone (**18b**) (0.74 g 1.38 mmol, 65%).^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.4 (s, 1H) 8.3 (*d*, 1H), 7.8 (*d*, 2H), 7.5 (*d*, 1H), 7.0 (*d*, 1H), 2.9 (*t*, 2H), 1.75 (q^5, 2H), 1.3 (q^5, 2H), 0.9 (*t*, 3H); *m/z*: 533 [$M+H^+$] was isolated as a yellow oil from **19a** (0.993 g, 2.13 mmol) and [$^2H_{18}$]-dibutylamine (total of 1.067 g, 7.24 mmol)

(2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylamino-1-[$^2H_6$]-propoxy)-phenyl]-methanone (**18d**) (1.50 g, 2.89 mmol, 80%) was isolated as a yellow oil from **19a** (1.69 g, 3.62 mmol) and **5** (0.964 g, 7.23 mmol). (2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-di-[$^2H_9$]-butylamino-propoxy)-phenyl]-methanone (**18f**) as a yellow oil (0.486 g, 0.92 mmol, 82%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.3 (s, 1H), 8.2 (*d*, 1H), 7.8 (*d*, 2H), 7.6 (*d*, 1H), 7.0 (*d*, 1H), 4.1 (*t*, 2H), 2.9 (*t*, , 2H), 2.6 (*t*, 2H), 1.8 (q^5, 2H), 1.75 (q^5, 2H), 1.4 (q^6, 2H), 0.9 (*t*, 3H). MS *m/z* 527 ($M+H^+$) from **19b** (0.993 g, 2.13 mmol) and [$^2H_{18}$]-dibutylamine (total of 0.812 g, 5.52 mmol)

(2-Butyl-5-nitro-benzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylaminopropoxy)phenyl]-methanone (**18h**) (0.661 g, 1.29 mmol, 80%) from **19b** (1.15 g, 2.50 mmol) and **5** (1.06 g, 7.95 mmol) characterised by comparison with analogues.

Synthesis of amines (20a-h) from nitro compounds (18a-h)

A solution of the nitro compound **18** in ethanol (about 35 volume equivalents) was treated with tin (II) chloride dihydrate (5 mol equivalents), and the mixture heated at 80°c under argon. When TLC indicated no starting material to remain, the mixture was quenched with ice, basified (5% aqueous sodium bicarbonate solution) treated with excess EtOAc and filtered over a Celite pad. After washing the filter cake with further EtOAc, the extract was washed with water, and the aqueous layer extracted twice with the same solvent. The combined organic phase was dried over Na_2SO_4 and evaporated. The resultant crude amines **20** were chromatographed in 98:2 dichloromethane-MeOH containing 0.2% concentrated ammonia solution over silica gel. By using this general method, the following were obtained:

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-dibutylamino-1-[$^2H_2$]-propoxy)-phenyl]-methanone (**20a**) (0.611 g, 1.27 mmol, 90%) from **18a** (0.715 g, 1.40 mmol) as a pale yellow oil.

(5-Amino-2-butylbenzofuran-3-yl)-[4-(3-di-[$^2H_9$]-butylamino-[$^2H_6$]-propoxy)-phenyl]-methanone (**20b**) (0.605 g, 1.20 mmol, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.85 (*d*, 2H), 7.3 (*m*, 1H), 6.95 (*d*, 2H), 6.6 (*m*, 2H), 3.55 (bs, 2H,), 2.85 (*t*, 2H), 1.75 (q^5, 2H), 1.3 (*m*, 2H), 0.9 (*t*, 3H). MS *m/z* 503 ($M+H^+$), from **18b** (0.711 g, 1.33 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-dibutylamino-[$^2H_6$]-propoxy)-phenyl]-methanone (**20c**) (0.404 g, 0.85 mmol, 95%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.85 (d, 2H), 7.3 (*m*, 1H), 6.95 (*d*, 2H), 6.6 (*m*, 2H), 3.55 (bs, 2H), 2.85 (*t*, 2H), 2.5 (*m*, 2 x 2H), 1.85 (*m*, 2H), 1.0 (*m*, 5x2H), 0.9 (*m*, 3 x 3H). MS *m/z* 503 ($M+H^+$) from **18c** (0.456 g, 0.89 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylamino-3-[$^2H_6$]-propoxy)-phenyl]-methanone (**20d**) (1.177 g, 2.27 mmol, 78%) from **18d** (1.50 g, 2.9 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-dibutylamino-3-[$^2H_2$]-propoxy)-phenyl]-methanone (**20e**) (0.51 g, 1.06 mmol, 72%) from **18e** (0.75 g, 1.47 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-di-[$^2H_9$]-butylaminopropoxy)-phenyl]-methanone (**20f**) (0.404 g, 0.81 mmol, 90%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.8 (*d*, 2H), 7.25 (*m*, 1H), 6.9 (*d*, 2H), 6.6 (*m*, 2H), 4.1 (*t*, 2H), 3.5 (bs, 2H), 2.9 (*t*, 2H), 2.6 (*t*, 2H), 1.8 (*m*, 2H), 1.75 (*m*, 2H), 1.3 (*m*, 2H), 0.9 (*t*, 3H). MS *m/z* 497 ($M+H^+$), from **18f** (0.473 g, 0.9 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylamino-3-[$^2H_2$]-propoxy)-phenyl]-methanone (**20g**) (0.33 g, 0.68 mmol, 58%) was obtained as a yellow oil from **19g** (0.75 g, 1.47 mmol)

(5-Amino-2-butyl-benzofuran-3-yl)-[4-(3-di-1-[$^2H_2$]-butylamino-propoxy)-phenyl]-methanone (**20h**) (643 mg, 1.33 mmol, 68%) from **18h** (1.00 g, 1.95 mmol).

[$^2H_4$]-N-{2-Butyl-3-[4-(3-dibutylamino-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (3h)

A solution of **20h** (0.500 g, 1.04 mmol) in dichloromethane (10 mL) was treated with triethylamine (210 μL, 1.5 mmol) and methanesulphonyl chloride (120 μL, 1.54 mmol) and stirred for 20 min at room temperature. The mixture was diluted with water and extracted three times with dichloromethane, the combined extracts were dried over $MgSO_4$ and evaporated to a beige foam shown by LC/MS analysis to consist of a 1:3 mixture of the required **3h**, and the disulphonylated compound (**21h**). The crude mixture was dissolved in 1:1 MeOH/THF (10 mL), transferred to a reaction flask and treated with 10% aqueous sodium carbonate solution (5 mL) and concentrated ammonia solution (5 mL). The reaction mixture was heated to reflux for 10 h, cooled and extracted three times with EtOAc. The combined extracts were dried over Na_2SO_4 and evaporated to a dark oil (500 mg). Column chromatography on silica gel in 98:2 dichloromethane/MEOH containing 0.2% concentrated aqueous ammonia gave **3h** free base (286 mg, 0.51 mmol, 49%) as an oil. This was taken up in 2-propanol, and treated with excess of a solution of HCl in 2-propanol. The solution was concentrated, treated dropwise with diethyl ether and refrigerated. The **3h** (0.22 g, 0.37 mmol, 35%) was recovered as a white solid by filtration, and dried under vacuum. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 12.06 (1H), 7.80 (*d*, 2H), 7.44 (*d*, 1H), 7.31 (dd, 1H), 7.18 (dd, 1H), 6.96 (*d*, 2H), 4.28 (bt, 2H), 3.24 (bt, 2H), 2.98 (*t*, 2H), 2.93 (s, 3H), 2.44 (*m*, 2H), 1.77-1.82 (*m*, 6H), 1.44-1.38 (*m*, 6H), 1.00 (*t*, 6H), 0.92 (*t*, 3H). MS *m/z* 561.3255 ($M+H^+$), 559.3171 ($M-H^-$).

Synthesis of methylsulphonamido hydrochlorides (3a-g) from amines (20a-h)

For the other compounds in the series, a solution of amine **20** in THF (about 20 volumes) and *t*-butylmethyl ether (about 10 volumes) was treated with methanesulphonyl chloride (1.0 molar equivalent) and 30% aqueous ammonia solution (1.0 molar equivalent). The mixture was stirred at room temperature, and if TLC indicated reaction to be incomplete, further methanesulphonyl chloride (1.0 molar equivalent) and 30% aqueous ammonia solution were added. Once reaction was complete, the mixture was partitioned between water and EtOAc, the layers separated and the aqueous phase re-extracted with EtOAc. The combined organic phase was dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel and converted to the hydrochloride salt by treating an ethereal solution with an anhydrous solution of HCl in ether. Hydrochloride salts were collected by filtration and dried under vacuum. By using this general method the following were synthesised:

N-{2-Butyl-3-[4-(3-dibutylamino-1-[$^2H_2$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3a**) (0.141 g, 21 %) as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.79 (*d*, 2H), 7.55 (bs, 1H), 7.44 (*d*, 1H), 7.32 (dd, 1H), 7.18 (*d*, 1H), 6.94 (*d*, 2H), 3.18 (dd, 2H), 3.06-3.00 (*m*, 6H), 2.39 (dd, 2H), 1.82-1.70 (*m*, 6H), 1.50-1.25 (*m*, 6H), 0.98-0.91 (*m*, 9H). MS *m/z* 559 ($M+H^+$); 557 [M-H-] from 20a (0.611 g, 1.27 mmol)

N-{2-Butyl-3-[4-(3-di-[$^2H_9$]-butylamino-[$^2H_6$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3b**) (0.205 g, 41%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 11.9 (bs, 1H), 7.8 (*d*, 2H), 7.55 (bs, 1H), 7.45 (*d*, 1H), 7.3 (*d*, 1H), 7.2 (s, 1H), 6.95 (*d*, 2H), 2.95 (*t*, 2H), 2.9 (s, 3H), 1.75 (*m*, 2H), 1.4 (*m*, 2H), 0.9 (*m*, 3H). MS *m/z* 581 ($M+H^+$) from **20b** (0.573 g, 1.14 mmol)

N-{2-Butyl-3-[4-(3-dibutylamino-[$^2H_6$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3c**) (0.480 g, 95%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 11.6 (bs, 1H), 7.9 (bs, 1H), 7.8 (*m*, 2H), 7.4 (*d*, 1H), 7.3 (*d*, 1H), 7.2 (s, 1H), 6.9 (*d*, 2H), 3.05 (bs, 4H), 2.95 (*t*, 3H), 2.9 (s, 3H), 1.75 (*m*, 9H), 1.4 (*m*, 6H), 0.95 (*t*, 6H), 0.9 (*t*, 3H). MS *m/z* 563 [M + H+] from **20c** (0.410 g, 0.85 mmol)

N-{2-Butyl-3-[4-(3-di-1-[$^2H_2$]-butylamino-[$^2H_6$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3d**) (0.17 g, 0.28 mmol, 14%) as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.79 (*d*, 2H), 7.44 (*d*, 1H), 7.31 (dd, 1H), 7.18 (*d*, 1H), 6.94 (*d*, 2H), 2.98 (*m*, 2H), 2.92 (s, 3H), 1.77-1.35 (*m*, 12H), 0.98-0.91 (*m*, 9H). MS *m/z* 567 ($M+H^+$), 565 ($M-H^-$) from **20d** (0.974 g, 2.0 mmol)

[$^2H_2$]-N-{2-Butyl-3-[4-(3-dibutylamino-3-[$^2H_2$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3e**) (0.48 g, 0.81 mmol, 86%) as a white solid from **20e** (0.51 g, 1.06 mmol). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.74 (*d*, 2H), 7.44 (d, 1H), 7.33 (dd, 1H), 7.18 (*d*, 2H), 4.26 (*t*, 3H), 3.15-2.95 (*m*, 6H), 2.93 (s, 3H), 2.41 (*t*, 2H), 1.90-1.70 (*m*, 6H), 1.50-1.30 (*m*, 6H), 0.99 (*t*, 6H). MS *m/z* 559 ($M+H^+$), 557 ($M-H^-$)

N-{2-Butyl-3-[4-(3-di-[$^2H_9$]-butylamino-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3f**) (0.205 g, 41%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 11.9 (bs, 1H), 7.8 (*m*, 3H), 7.4 (*d*, *J* = 12 Hz, 1H), 7.3(*d*, *J* = 6 Hz, 1H), 7.2 (s, 1H), 6.9 (*d*, *J* = 6 Hz, 2H), 4.2 (*m*, 2H), 3.2 (bs, 2H), 2.95 (*t*, 3H), 2.9 (s, 3H), 2.4 (bs, 2H), 1.8 (*m*, 2H), 1.35 (*m*, 2H), 0.9 (*m*, 3H). MS *m/z* 575 ($M+H^+$) from **20f** (0.404 g, 0.81 mmol)

N-{2-Butyl-3-[4-(3-di-1-[$^2H_2$]-butylamino-3-[$^2H_2$]-propoxy)-benzoyl]-benzofuran-5-yl}-methanesulfonamide; hydrochloride (**3g**) (0.270 g, 0.53 mmol, 78%) as a white solid from 20g (0.33 g, 0.68 mmol) ^{1}H NMR ($CDCl_3$, 600 MHz) δ 11.76(bs, 1H), 7.78(*d*, 2H), 7.43(*d*, 1H), 7.38(dd, 1H), 7.19(*d*, 1H), 6.93(*d*, 1H), 4.23(*t*, 2H), 2.96(*t*, 2H), 2.92(s, 3H), 2.40(*t*, 2H), 1.79-7.74(*m*, 6H), 1.50-1.35(*m*, 6H), 0.98 (*t*, 6H). MS m/z) 563 ($M+H^+$), 561 ($M-H^-$)

Conclusion

Site specific deuteration of dronedarone in a variety of positions in the metabolically labile part of the molecule had little to no significant effect on the *in vitro* behaviour of the parent compound in the human hepatocyte model. Overall metabolic clearance of dronedarone is nearly exclusively CYP-dependent and is dominated by CYP3A4 dependent *N*-debutylation. These results confirm previous findings, for example the low (<2) kinetic isotope effect observed for P450 mediated demethylation of N,*N*-dimethylaniline,[17], which is explained by invoking a single electron transfer mechanism—although this remains a subject of much debate.[18] This observation may be of help in determining which substrates have a greater chance of exhibiting reduced rates of metabolic clearance once deuterated.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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