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Effect of deuteration on metabolism and clearance of Nerispirdine (HP184) and AVE5638

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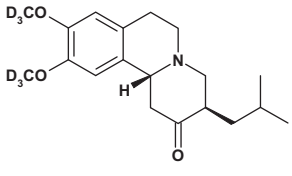
ABSTRACT

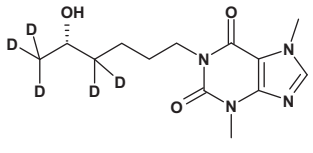
Replacing hydrogen with deuterium as a means of altering ADME properties of drug molecules has recently enjoyed a renaissance, such that at least two deuterated chemical entities are currently in clinical development. Although most research in this area aims to increase the metabolic stability, and hence half-life of the active species, experience has shown that prediction of the in vivo behaviour of deuterated molecules is difficult and depends on multiple factors including the complexity of the metabolic scheme, the enzymes involved and hence the mechanism of the rate-determining step in the biotransformation. In an effort to elucidate some of these factors we examined the metabolic behaviour of two molecules from the Sanofi portfolio in a range of in vitro and in vivo systems. Although some key metabolic reactions of the acetylcholine release stimulator HP184 **4** were slowed in vitro and in vivo when deuterium was present at the sites of metabolism, this did not translate to an increase in overall metabolic stability. By contrast, the tryptase inhibitor AVE5638 **13** was much more metabolically stable in vitro in its deuterated form than when unlabelled. These results indicate that it could be of value to concentrate efforts in this area to molecules which are metabolised by a major pathway that involves enzymes of the amine oxidase family or other low-capacity enzyme families.

1. Introduction

Of the various chemical modifications used in medicinal chemistry to alter ADME properties of drug candidates, replacement of hydrogen by deuterium has recently received much attention as a way to prolong half-lives of rapidly metabolised drug molecules.[1,2] Although these attempts have a long history,[3] no deuterated molecule has yet made it to market. The recent upsurge of activity in this area has, however, resulted in a candidate molecule, SD-809 **1** reaching Phase III clinical trials,[4] with further examples, including CTP-499, **2**,[5] also in the clinic.



1: SD809



2: CTP-499

The theoretical principle underlying these attempts, that of the kinetic isotope effect (KIE), relies on the assumption that a metabolic reaction which breaks a C–H bond in the rate-determining step will be slowed down when hydrogen is substituted for deuterium.[6,7] Depending on the mode of action, this could translate to increased exposure to the parent, decreased formation of a toxic or reactive metabolite, or switching to a metabolic pathway which enhances formation of a species with increased activity.[7] It has become increasingly clear that this simple rationale is difficult to put into practice in vivo: the vast majority of metabolic reactions responsible for clearance are mediated by one or several enzymes of the cytochrome P450 family which present such a wide range of reactivities, that simply slowing one pathway may not result in appreciable alteration of the ADME property being targeted.[7] Additional complicating factors have also been discussed by authors in the field.[8]

In order to better understand the scope and limitations of applying the deuterium isotope effect to real cases in pharmaceutical development and to attempt to understand which structural and biochemical factors are important in translating the theory into practical examples, we have undertaken a selective study of

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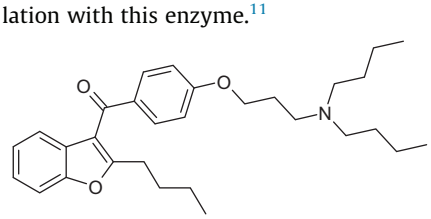
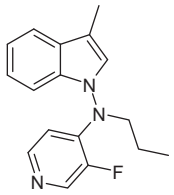
some compounds from the Sanofi portfolio and attempted to gauge the effect that site-specific deuteration has on their metabolism and clearance. Our recently published work on dronedarone, **3**,[9] a compound whose in vitro metabolism is dominated by CYP3A4-mediated N-debutylation[10] showed that deuteration in a variety of metabolised positions had essentially no effect on the metabolic stability of the compound. This confirmed previous findings that deuteration can often have little effect on the kinetics of N-dealkylation with this enzyme.[11]



3: dronedarone

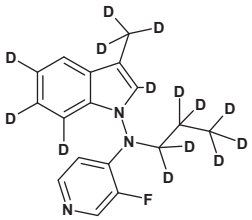


4: HP184, nerispirdine

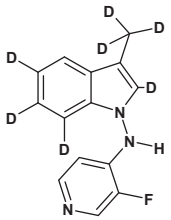
HP184 (Nerispirdine **4**) has been developed as an acetylcholine release stimulator for treatment of spinal cord injury.[12] It is extensively metabolised in vitro, in animals, and in humans. **4** is metabolised principally by N-dealkylation to secondary amine HP183 **5**, and thence by oxidation to the carboxylic acid HP185 **9** which is recovered in part as its glucuronide, **12**. Carboxylic acid **8**, with the propyl group intact, is also recovered as its glucuronide **11**.[13] Furthermore, formation of both carboxylic acids occurs after prior hydroxylation of the methyl group. In addition to these major pathways, several other metabolic routes have been observed in the different experiments, the most important of which begins with aromatic hydroxylations to the phenols **10a** and **10b** (see Scheme 1).[13]

In vitro experiments using ^{14}C-labelled **4** incubated with human liver microsomes have shown that the N-dealkylation pathway is primarily mediated by CYP3A4, but that isoforms 1A2 and 2D6 are implicated to a minor extent. Additionally, the methyl hydroxylation which begins the pathway to the acids is mediated by many different isoforms including 1A2, 2C9, 2C19 and 2D6, although it was not possible to quantify the individual contributions.[13]

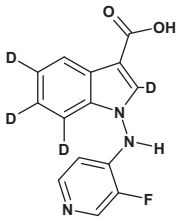
In order to study the effect of deuteration on the rate of metabolism, we targeted a derivative carrying deuterium atoms at each of these three major sites of metabolism, namely the fused phenyl ring, the propyl side chain and the indole 3-methyl group. **4a** was thus synthesised and its metabolic behaviour studied in vitro using human hepatocytes and microsomes, and in vivo in rats and compared to that of **4**, by following the rate of clearance of **4** versus **4a** and the comparative kinetics of formation of metabolite **5** versus **5a** and **9** versus **9a**.



4a: HP184-d_{14}

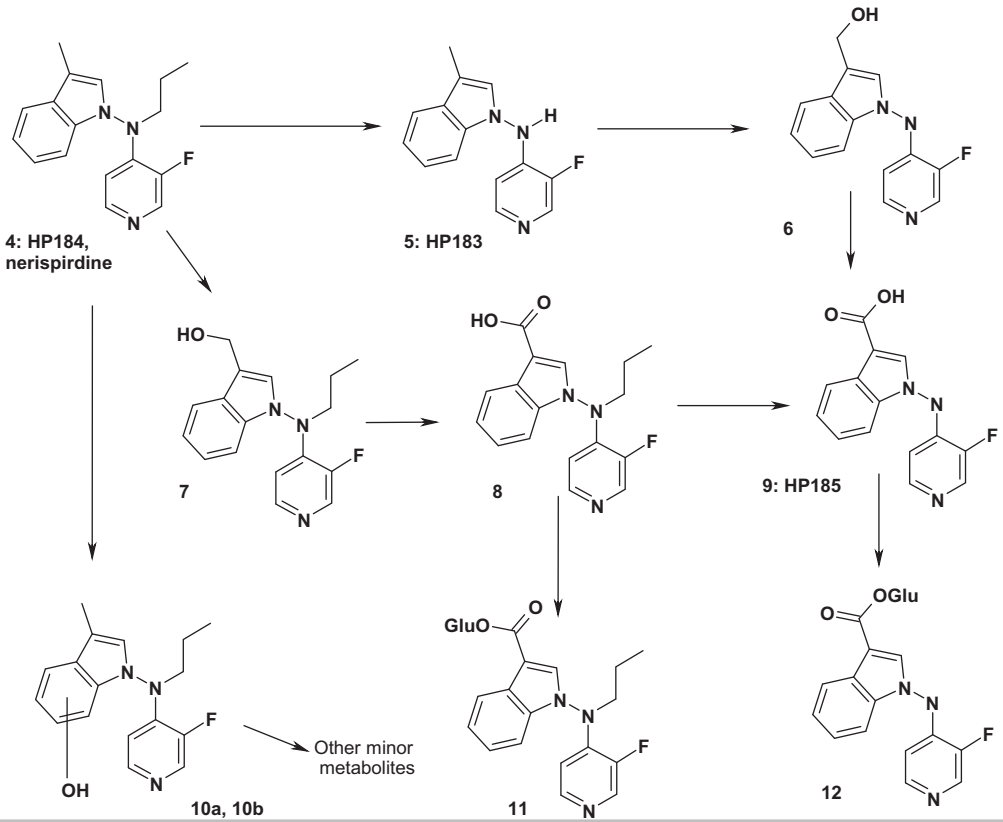


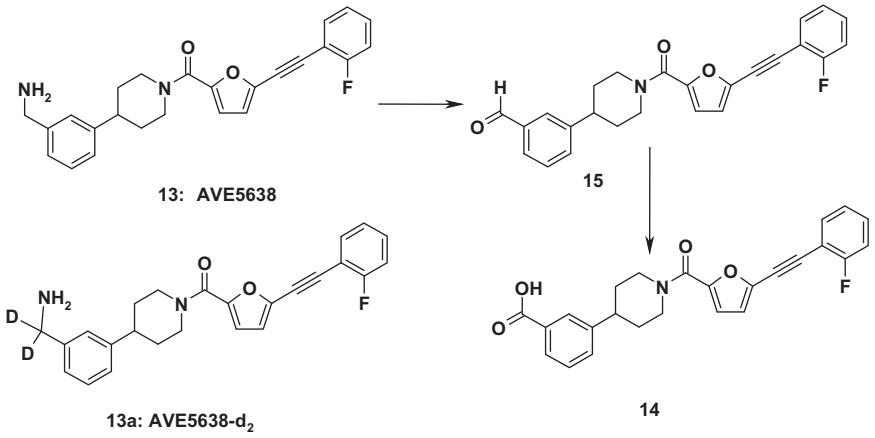
5a: HP183-d_7



9a: HP185-d_4

AVE5638,[14] **13** was developed as a tryptase inhibitor, and its metabolism is dominated by oxidative deamination to the carboxylic acid **14** via aldehyde **15**. In common with other compounds of this class[15] and β tryptase itself, this transformation is mediated by semicarbazide sensitive amine oxidase (SSAO)[16] (see Scheme 2).





Scheme 2. Oxidative metabolism of AVE5638, **13** to carboxylic acid, **14** and structure of deuterated analogue, **13a**.

To investigate the effect of deuteration on this single, non-CYP metabolic clearance pathway, we prepared a deuterated derivative, **13a** carrying D atoms at the site of oxidative deamination. Its rate of metabolism was compared to that of its unlabelled parent, **13** in human liver subcellular fractions (S9 and microsomes) and in human hepatocytes.

2. Methods and results

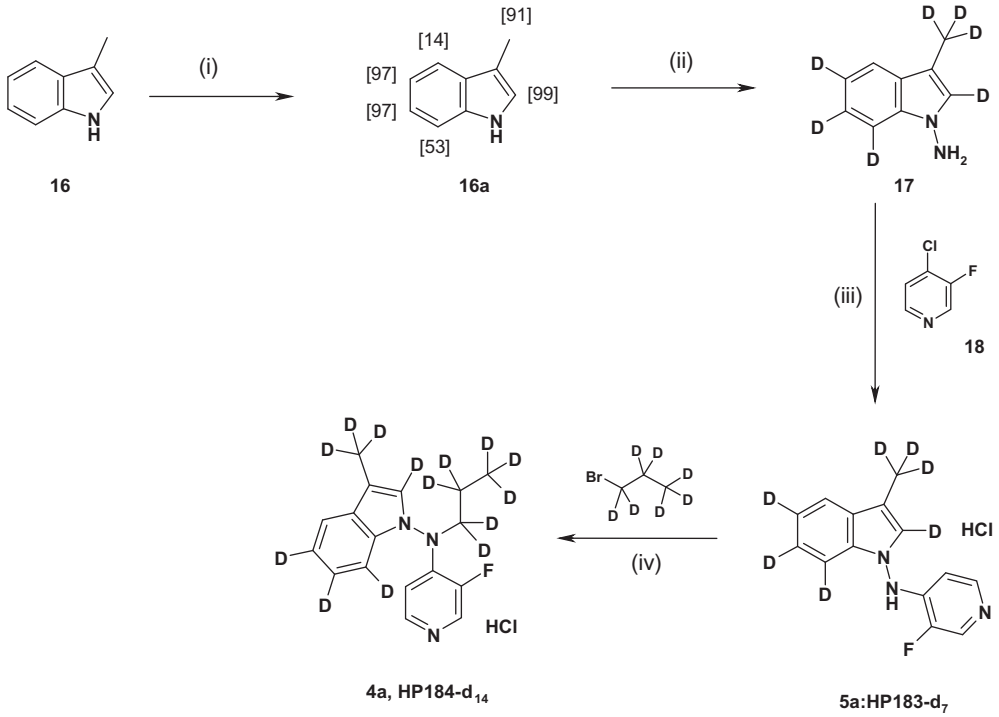
2.1. Chemistry

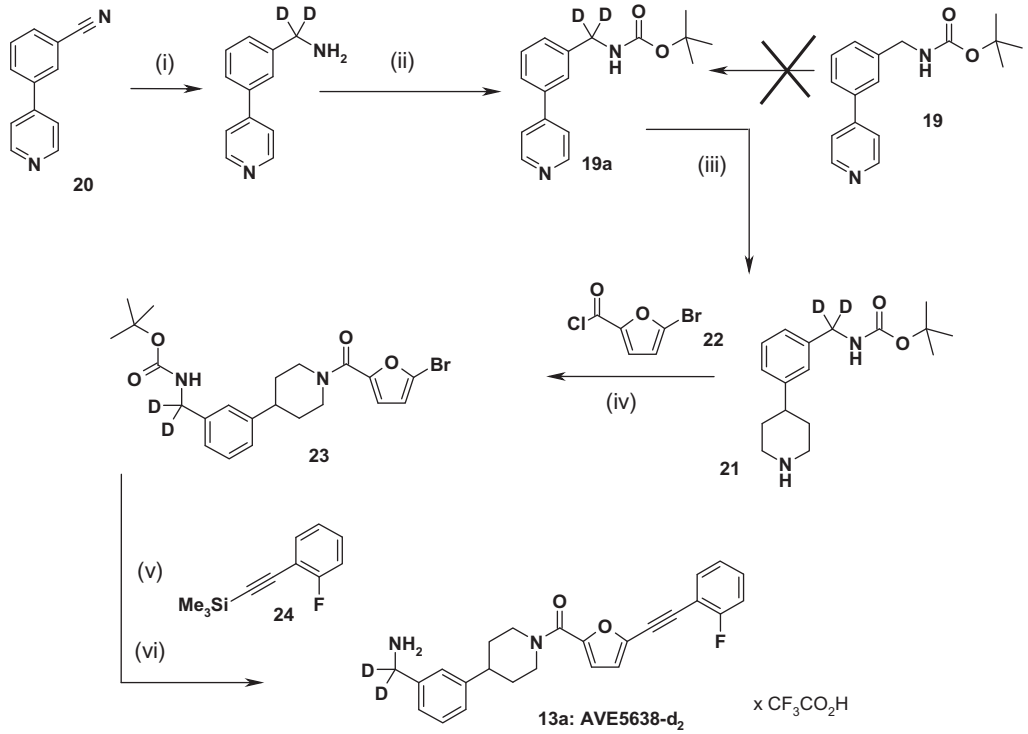
2.1.1. HP184

Recent work in our group[17] and others active in the field[18] has shown the versatility of hydrogen-deuterium exchange reactions to prepare deuterated derivatives, and in the case of 3-methylindole **16**, use of a mixed platinum–palladium catalyst system under microwave enhanced conditions, led to excellent deuterium incorporation levels at most positions in **16a**. For the purpose of this study we aimed to exchange as many H atoms for D as possible, but the aromatic protons at the 4 and 7 positions were resistant to these conditions. For convenience, any positions exchanged to greater than 50% are shown as deuterated (Scheme 3). Using an adaptation of conditions described in the literature, the deuterated intermediate **16a** was then *N*-aminated using methodology developed in our laboratories[19] and the resultant *N*-amino compound **17** reacted with 3-chloro-2-fluoropyridine **18** giving a good yield of **5a**. N-alkylation with commercially available *n*-1-bromopropane-d_7 under microwave irradiation, followed by reverse-phase HPLC gave the target compound **4a** in 28% overall yield, using previously described conditions.[20]

2.1.2. AVE5638

Surprisingly, the major challenge in the synthesis of **13a** was the selective deuteration at the benzylic position. Attempts to





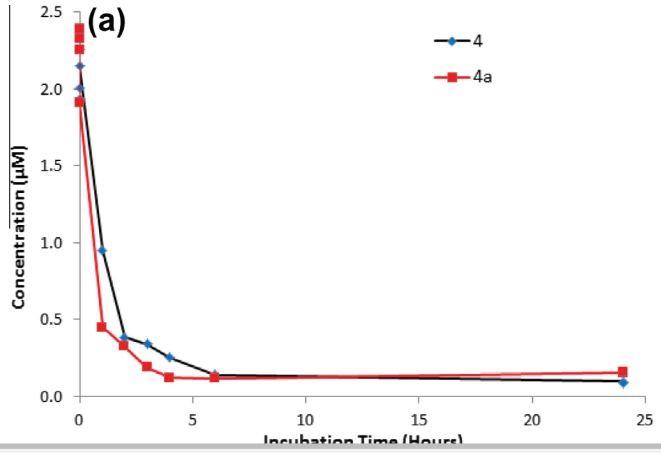
Scheme 4. Synthesis of AVE5638-d_2 **13a**. Reagents (i) $LiAlD_4/AlCl_3$; (ii) Boc_2O, 30%, 2 steps; (iii) H_2, Pt–C, HCl, EtOH, 77%; (iv) **22**, Et_3N, toluene, 66%; (v) **24**, CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, THF; (vi) $MeSO_3H$, followed by preparative HPLC with TFA, 78%.

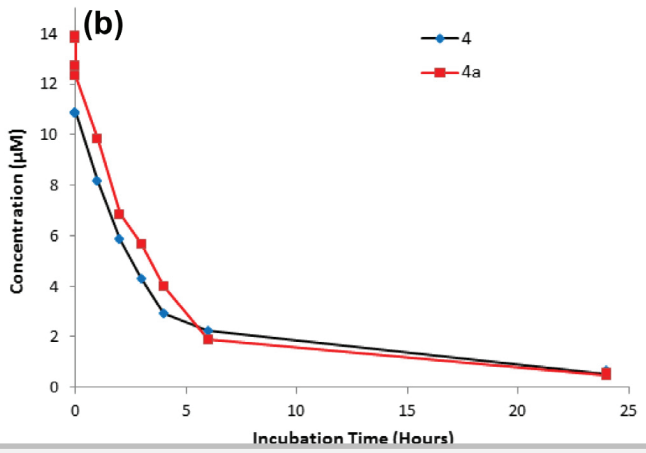
follow the procedure of Greene et al.[21] via formation of the benzylic dianion of the N-Boc benzylamine **19** followed by a D_2O quench, yielded mixtures of products in which deuterium incorporation was incomplete. A second approach, in which nitrile **20** was reduced using $LiAlD_4$ under standard conditions[22] also failed to give workable amounts of the required deuterated amine. Attempts with $NaBD_4/CoCl_2$,[23] $NaBD_4/NiCl_2$[24] or variations using $LiAlD_4$[25] were likewise disappointing. Finally, we succeeded in selectively reducing **20** with lithium aluminium deuteride in presence of aluminium chloride,[26] followed immediately by Boc protection, to give the deuterated carbamate **19a**. **19a** was converted to the target compound using a modification of published methods.[27] Thus, partial reduction to the tetrahydropyridine, **21** followed by reaction with substituted furoic acid chloride, **22** gave amide **23** in good yield. **23** was reacted under modified Sonogashira conditions with the acetylene, **24** to give **13a** as its free base, which was converted to its trifluoroacetate after purification (see Scheme 4). The overall yield of the synthesis was 10%.

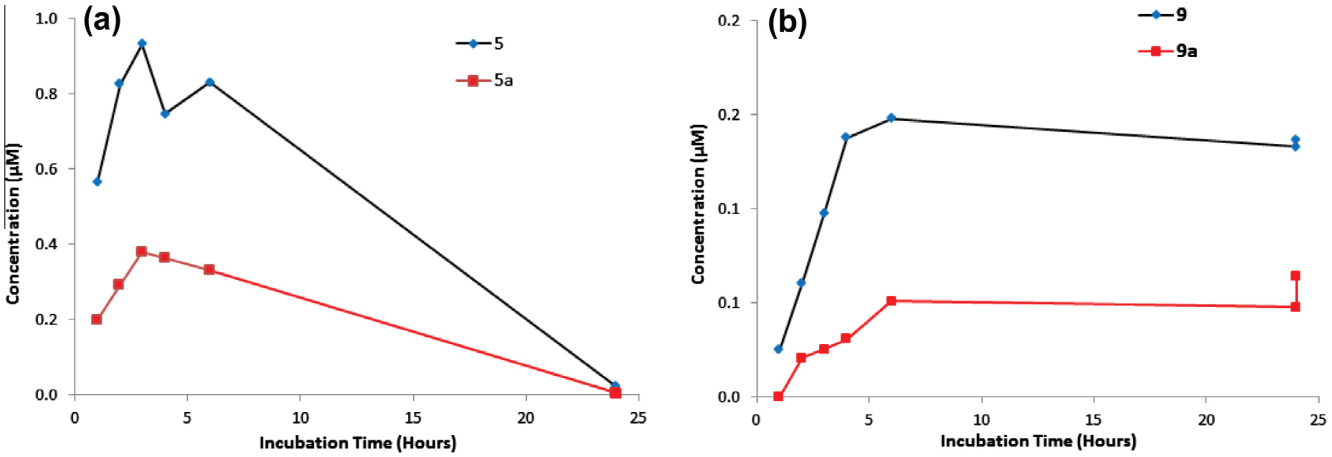
2.2. Metabolism studies

2.2.1. HP184 in vitro

The in vitro metabolism of **4a** was examined and compared to that of the unlabelled compound **4** following incubation with two different preparations of plated human hepatocytes at concentrations of 2 and 10 μM. Aliquots were withdrawn at various time points up to 24 h and, analysed by LC–MS/MS for parent compounds (**4**/**4a**), the *N*-depropylated metabolites (**5**/**5a**) and carboxylic acid metabolites (**9**/**9a**). There was no significant difference in the results observed between the two preparations. The time-course of the metabolism experiment with respect to the concentration of the parent compound and the two metabolites are shown in Graphs 1 and 2 for one hepatocyte preparation.



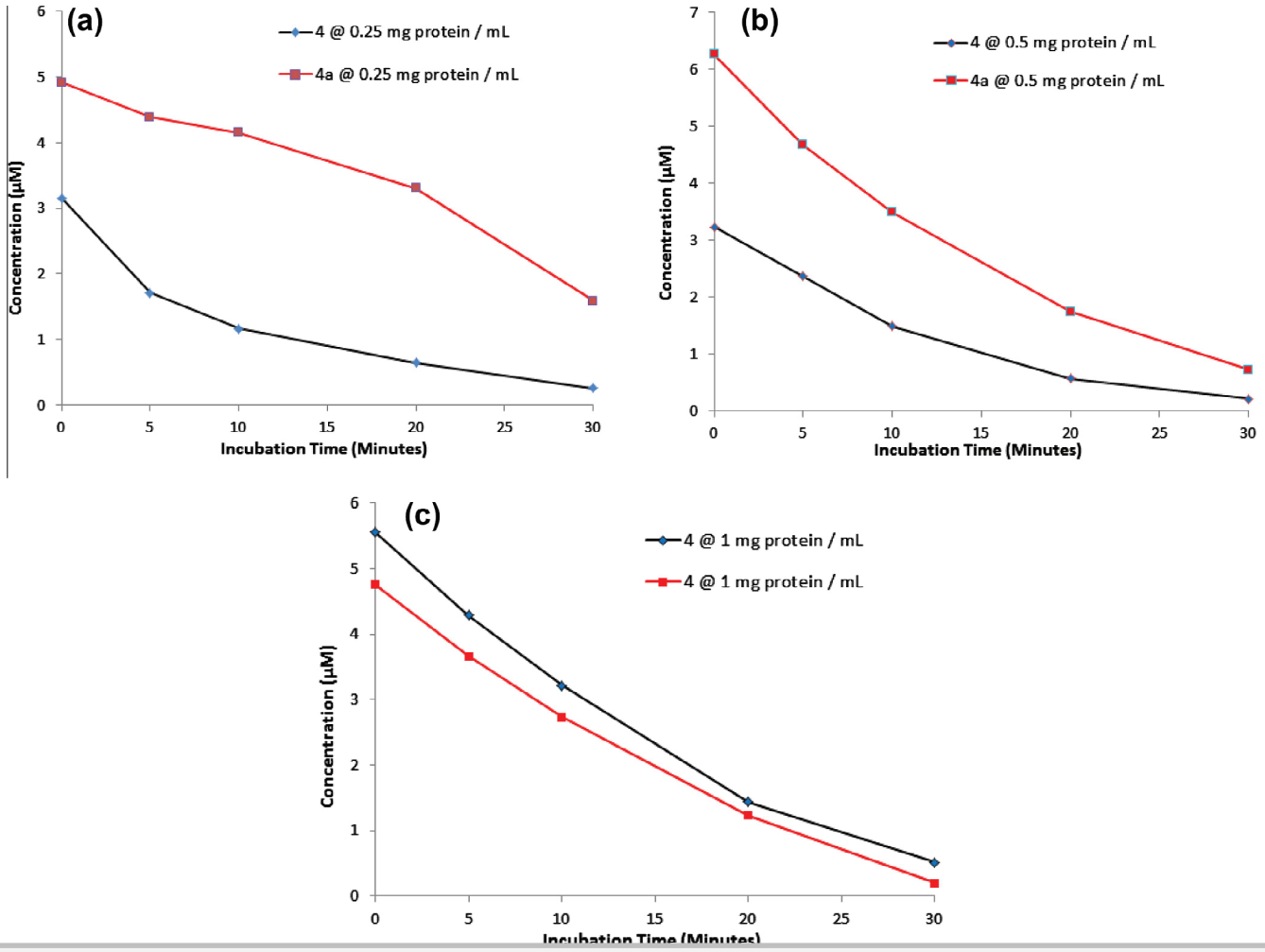




Graph 2. Time versus concentration of (a) **5**/**5a** and (b) **9**/**9a** in incubation of 10 μM **4**/**4a** in presence of plated human hepatocytes.

Additionally, similar comparative experiments were conducted in human liver microsomes at a substrate concentration of 5 μM, at protein concentrations of 0.25, 0.5 and 1 mg of protein/mL. Aliquots were withdrawn at various time points up to 30 min and, analysed by LC–MS/MS for parent compounds (**4**/**4a**), the *N*-depropylated metabolites (**5**/**5a**). Note that the carboxylic acid metabolites (**9**/**9a**) were not formed in these incubations.

The time-course of the metabolism experiments with respect to the concentration of the substrate and the metabolites of the three compounds at the various protein concentrations are shown in Graphs 3 and 4.



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