1521-009X/45/11/1133–1138$25.00
Drug Metabolism and Disposition

https://doi.org/10.1124/dmd.117.077776
Drug Metab Dispos 45:1133–1138, November 2017

CYP-Mediated Sulfoximine Deimination of AZD6738

Barry C. Jones, Roshini Markandu, Chungang Gu, and Graeme Scarfe

Oncology IMED, AstraZeneca, Cambridge, United Kingdom (B.C.J., R.M., G.S.); and Oncology IMED, AstraZeneca, Waltham, Massachusetts (C.G.)

Received July 27, 2017; accepted August 18, 2017

ABSTRACT

In hepatic S9 and human liver microsomes (HLMs) the sulfoximine moiety of the ATR inhibitor AZD6738 is metabolized to its corresponding sulfoxide (AZ8982) and sulfone (AZ0002). The initial deimination to AZ8982 is nominally a reductive reaction, but in HLMs it required both NADPH and oxygen and also was inhibited by 1-aminobenzotriazole at a concentration of 1 mM. Studies conducted in a panel of 11 members of the cytochrome P450 (P450) family (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 CYP2J2, CYP3A4, and CYP3A5) confirmed that deimination was an oxidative process that was mediated largely by CYP2C8 with some CYP2J2 involvement, whereas the subsequent oxidation to sulfone was carried out largely by CYP2J2, CYP3A4, and CYP3A5. There was no measureable metabolism in flavin-containing monooxygenase (FMO) enzymes FMO3, FMO5 or NADPH cytochrome C reductase. Studies using Silensomes, a commercially available HLM in which specific members of the P450 family have been inhibited by selective mechanism-based inhibitors, showed that when CYP2C8 was inhibited, the rate of deimination was reduced by 95%, suggesting that CYP2J2 is only playing a minor role in HLMs. When CYP3A4 was inhibited, the rate increased by 58% due to the inhibition of the subsequent sulfone formation. Correlation studies conducted in HLM samples from different individuals confirmed the role of CYP2C8 in the deimination over CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A. Hence, although nominally a reduction, the deimination of AZD6738 to its sulfoxide metabolite AZ8982 is an oxidation mediated by CYP2C8, and this metabolite is subsequently oxidized to the sulfone (AZ0002) largely by CYP3A.

Introduction

Ataxia telangiectasia–mutated and RAD3-related protein is a serine/threonine protein kinase that forms part of the DNA-damage response coordinating the cellular response to DNA damage, stress, and cell-cycle perturbation (Cimprich and Cortez, 2008) and has become an attractive therapeutic target in cancer therapy (Foote et al., 2015; Weber and Ryan, 2015). AZD6738 (Fig. 1) is a selective and potent inhibitor of ataxia telangiectasia–mutated and RAD3-related protein, is orally active and bioavailable (Guichard et al., 2013; Jones et al., 2013), and has been taken into clinical development (ClinicalTrials.gov Identifiers: NCT02223923; NCT02630199; NCT01955668; and NCT02264678).

From a structural and metabolic perspective, the key functional group of interest is the sulfoximine moiety. A recent review by Sirvent and Lucking (2017) has highlighted that sulfoximines are being increasingly used in drug discovery as well as in clinical candidates. Beyond AZD6738, roniciclib and BAY 1143572 both contain sulfoximine moieties. The authors point out that sulfoximines have a unique set of physicochemical and hydrogen-bonding properties that make it a moiety of utility for medicinal chemistry and show this by replacing either secondary or tertiary amines with sulfoximines into a set of clinical compounds and comparing pharmacological, physicochemical, and drug metabolism properties. The sulfoximine analogs had similar pharmacological potencies and lipophilicities despite an increased degree of hydrogen bonding. This work highlights that although the drug analogs that were made showed generally better metabolic stabilities, there is little known about the metabolism of sulfoximines. Roniciclib is in clinical trials as a pan-CDK inhibitor with reportedly favorable pharmacokinetic properties in preclinical species, but to date there is no published metabolism information on this compound (Bahleda et al., 2017).

Buthionine sulfoximine is an inhibitor of glutamylcysteine synthetase and is used in cancer chemotherapy to deplete glutathione and so make cells vulnerable to reactive oxygen species (Glasauer and Chandel, 2014). Despite many years of clinical use, there is very little information on its metabolism. The information that does exist shows that the compound is excreted largely unchanged in the urine with a small amount of an acylated metabolite also being formed (Ahluwalia et al., 1990). Thus, there is no information on the metabolic fate of the sulfoximine.

Once a metabolic route has been defined, reaction phenotyping studies are now routinely carried out to define what proportion of the route is mediated by different enzymes. Generally these studies comprise a number of different parts which can include inhibition with specific inhibitors, metabolism by recombinant enzymes and correlation with specific probe substrate activities across a panel of different biologic samples (Zientek and Youdim, 2015). This approach has been used to understand the contribution of CYP and non CYP enzymes to metabolic transformations (Argikar et al., 2016).

The aim of the current study was to characterize the in vitro metabolism of AZD6738, particularly as it relates to the sulfoximine moiety and to conduct reaction phenotyping studies to understand the fate of this group and the enzymes involved.

Materials and Methods

Materials. Compounds AZD6738, AZ8982 (sulfoxide), and AZ0002 (sulfone) were synthesized and developed at AstraZeneca UK Ltd. (Cambridge, UK). Pooled (150 donors; equal mix of sexes) human liver microsomes (HLMs), individual donor HLMs, and recombinant flavin-containing monooxygenases

All authors involved are employees of AstraZeneca.
https://doi.org/10.1124/dmd.117.077776.

ABBREVIATIONS: ABT, 1-aminobenzotriazole; ACN, acetonitrile; CLint, intrinsic clearance; FMO, flavin-containing monooxygenase; HLM, human liver microsomes; K_m, Michaelis constant; P450, cytochrome P450.

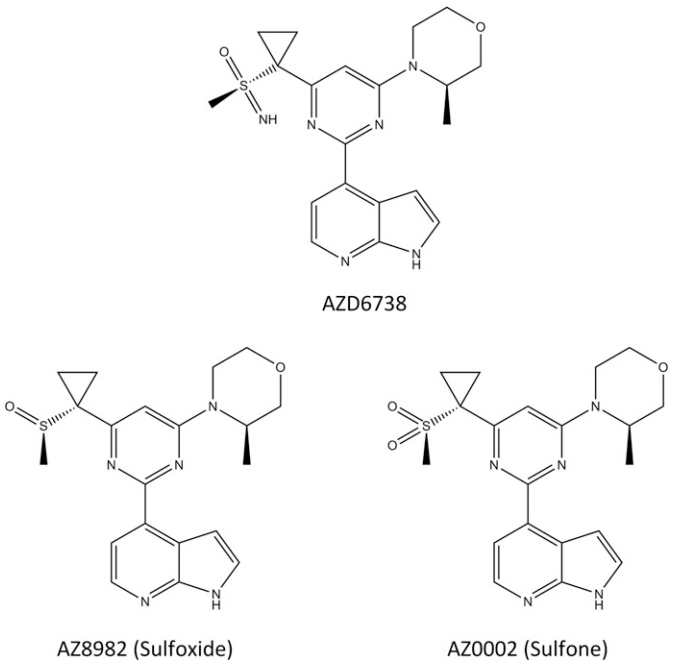

Fig. 1. Structures of AZD6738 and its sulfoxide (AZ8982) and sulfone (AZ0002) metabolites.

(FMOs; FMO3 and FMO5) were obtained from BD Biosciences (Oxford, UK). Human liver S9 fractions (mixed sex) were obtained from BioreclamationIVT (Baltimore, MD). Recombinant members of the cytochrome P450 (P450) family (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, and CYP3A5) and human reductase bactosomes (human NADPH P450 reductase expressed in *Escherichia coli*) were obtained from CYPEX (Dundee, UK). Silensomes were obtained from Biopredic International (Saint Grégoire, France). NADPH, dimethylsulfoxide, ketoconazole, 1-aminobenzotriazole (ABT), *N*-desethylamodiaquine, amodiaquine, midazolam, 1′-OH midazolam, phenacetin, paracetamol, diclofenac, 4′-OH diclofenac, bufuralol, hydroxybufuralol, *S*-mephenytoin, and 4-OH mephenytoin were purchased from Sigma-Aldrich (Poole, UK). Methanol, water, and acetonitrile (ACN) were high-performance liquid chromatography grade and purchased from Sigma-Aldrich.

Microsomal, S9, Cytosol, and Recombinant Enzyme Incubation Conditions. Metabolic studies in human liver S9 fractions (1 mg/ml), cytosol (1 mg/ml), HLM (0.5 mg/ml); and studies of recombinant members of the P450 family (100 pmol/ml), recombinant FMOs (0.25 mg/ml), and NADPH P450-reductase bactosomes (100 pmol/ml) were conducted in the presence of 1 mM NADPH, mixed with 0.1 M phosphate buffer, pH 7.4, and warmed to 37°C on a temperature-controlled heater block. Experiments were initiated by the addition of test compounds to give a 1 μM nominal test compound concentration in the incubation. One millimolar ABT was used in addition to the above conditions in the HLM incubations for the determination of P450 contribution.

The data were analyzed using a linear fit of the natural logarithm of the ratio of the compound peak area to the internal standard peak area against time. Intrinsic clearance (CLint) values were then calculated from the negative slope of the linear fit divided by the protein concentration. All the incubations were carried out in duplicate.

HLM Incubations Under Anaerobic Conditions. The 0.1 M phosphate buffer in capped vials on ice was bubbled with nitrogen for 30 minutes. Next, 495 μl of incubation mixture containing HLM (0.5 mg/ml) and NADPH (1 mM) was added to the vials, and the vials were capped and bubbled gently with nitrogen for a further 5 minutes on ice. The reaction was initiated by the addition of 5 μl of substrate (final concentration, 1 μM), the vials were capped, placed on a 37°C heater block, and bubbled with nitrogen to maintain an anaerobic atmosphere. Control incubations under aerobic conditions were carried out alongside. All the incubations were carried out in duplicate.

HLM Enzyme Kinetics Incubation. In time and protein linearity experiments, AZD6738 was incubated at 1 and 5 μM containing 1 mM NADPH and 0.1, 0.25, 0.5, and 1 mg/ml HLM in 0.1 M phosphate buffer, pH 7.4, for 0, 5, 10, 20, 40, and 60 minutes at 37°C. After this, AZD6738 was incubated at 200, 150, 100, 75, 50, 25, 10, 7.5, 5, 2.5, 1, and 0.5 μM with 1 mM NADPH and 0.5 mg/ml HLM in 0.1 M phosphate buffer, pH 7.4, for 30 minutes at 37°C. This study was carried out in duplicate. Data were analyzed in GraphPad Prism (GraphPad Software, San Diego, CA) to obtain the Michaelis constant (K_m) and V_{max} values.

Incubations with Silensomes. AZD6738 was incubated at a concentration of 25 μM with 1 mM NADPH and 0.5 mg/ml protein mixed in 0.1 M phosphate buffer, pH 7.4, for 30 minutes at 37°C. Positive controls for each P450 enzyme was assayed alongside AZD6738 to confirm the viability of the Silensomes product (data not shown). All incubations were carried out in triplicate.

Correlation Analysis. AZD6738 (25 μM), amodiaquine (20 μM), midazolam (3 μM), diclofenac (10 μM), *S*-mephenytoin (35 μM), phenacetin (30 μM), and bufuralol (5 μM) were incubated with HLMs from seven individual donors along with the pooled HLMs, all at 0.5 mg/ml, with 1 mM NADPH in 0.1 M phosphate buffer, pH 7.4, for 30 minutes at 37°C. All incubations were carried out in duplicate.

Sample Processing and Analysis. The experiments above were initiated by the addition of test compounds. Twenty-five microliters of incubates were removed into 100 µl of ACN containing internal standard per time point (0, 5, 10, 20, 40, and 60 minutes), unless stated otherwise. The samples were then centrifuged at 3000 rpm for 10 minutes, and 50 µl of supernatant was diluted with 300 μl of water before liquid chromatography-tandem mass spectrometry analysis. For all experiments, sulfoxide and sulfone calibration curves were generated in the relevant matrices with a limit of detection for both the sulfoxide and sulfone of 1 nM. In all experiments, the final solvent concentration was always $<$0.1% dimethylsulfoxide and $<$1% ACN.

Instrumentation. All liquid chromatography-tandem mass spectrometry data were acquired on a Xevo TQ-S Mass Spectrometer (Waters, Milford, MA) connected to a ACQUITY UPLC System (Waters). The Waters ACQUITY System consisted of an autosampler, binary ultra-performance liquid chromatography pump, column oven, autoinjector, and photodiode array detector. The analytes were separated by reverse-phase liquid chromatography using a KINETEX C18 column (50 $\times$ 2.1 mm, 2.6 μm; Phenomenex, Macclesfield, UK) preceded by a guard filter in a column oven at 50°C. The mobile phase consisted of high-performance liquid chromatography grade water with 0.1% formic acid (eluent A) and methanol with 0.1% formic acid (eluent B). The elution profile was as follows: linear gradient, 95% A to 5% A, 0.00–2.2 minutes; isocratic hold, 5% A, 2.2–2.6 minutes; and re-equilibration, 95% A, 2.61–2.8 minutes. The flow rate was 0.6 ml/min, and the eluent was introduced into the mass spectrometer via the divert valve at 0.5 minutes. The injection volume was 3 µl. The Xevo TQ-S Mass Spectrometer (Waters) was equipped with an electrospray ionization source, which was operated in positive mode. The mass

TABLE 1

Mass spectrometer parameters for AZD6738, AZ8982 (sulfoxide), and AZ0002 (sulfone)

Analyte	AZD6738	AZ8982	AZ0002
MRM m/z (Parent → Daughter)	413.1352 > 334.2479	397.9886 > 335.2584	414.1475 > 264.2618
Dwell (s)	0.056	0.056	0.056
Cone Voltage (V)	40	10	10
Collision Energy (V)	28	22	40

MRM, multiple reaction monitoring.

spectrometer source settings were as follows: the capillary voltage was 0.7 kV and the source offset was 59 V. The desolvation temperature was set to 600°C. Nitrogen was used as the desolvation gas (1000 l/h) and cone gas (150 l/h). Argon was used as the collision gas at a flow rate of 0.15 ml/min. Detection of the ions was performed in the multiple reaction monitoring mode using the transitions described in Table 1. Peak integration and calibrations were performed using TargetLynx software (version 4.1; Waters).

The structures of AZD6738, the sulfoxide metabolite AZ8982, and the sulfone metabolite AZ0002 are shown in Fig. 1.

Results

Preliminary Characterization. AZD6738 was incubated in different human hepatic S9 cytosol and microsomal fractions at a concentration of 1 μM to determine the nature of the enzyme mediating the deimination (Fig. 2).

In S9 fractions, AZD6738 was metabolized to the sulfoxide (AZ8982) and the sulfone (AZ0002) in the presence of NADPH. In the absence of NADPH, there was no apparent formation of either of these metabolites. In the presence of NADPH, the rate of formation of AZ133682982 was 0.3 pmol/min/mg, whereas the rate for AZ0002 was lower at 0.16 pmol/min/mg (Fig. 2).

There was no apparent formation of either metabolite in cytosol, ruling out the involvement of soluble enzymes such as aldehyde and xanthine oxidase.

When AZD6738 (1 μM) was incubated with HLMs in the presence of NADPH, the formation of both AZ8982 and the corresponding sulfone AZ0002 was observed. In the absence of NADPH, there was no measureable formation of either metabolite. Similarly, under anaerobic conditions there was no measurable formation of either metabolite compared with control incubations carried out under aerobic conditions. Preincubation with 1 mM ABT under aerobic conditions significantly reduced the rate of formation of both AZ8982 and AZ0002 by more than 80% for both metabolites (Fig. 2).

These preliminary studies strongly suggested that the deimination was not a reductive process but rather an oxidative one mediated by enzymes in the microsomal fraction such as either P450 family members or FMOs.

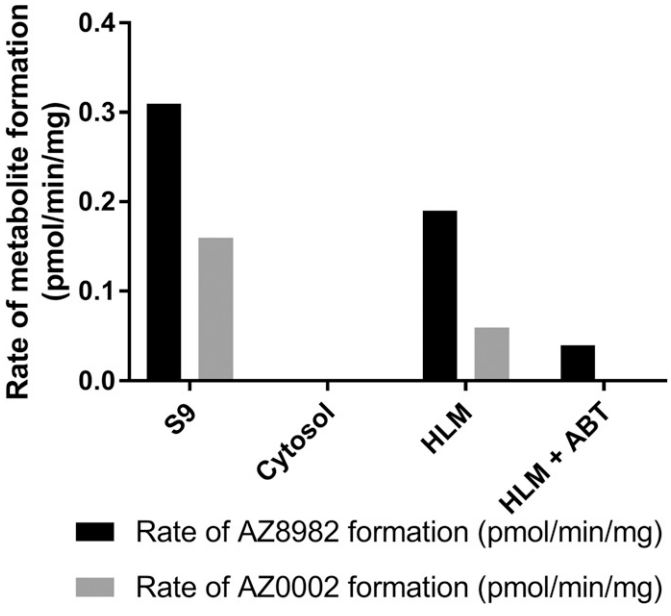

Fig. 2. Rate of formation of AZ8982 and AZ0002 in human hepatic S9, cytosol and microsomes.

HLM Enzyme Kinetics. The enzyme kinetics of the deimination of AZD6738 was investigated in pooled HLMs by following the rate of formation of AZ8982 over an AZD6738 concentration range. Initial studies in a 1 μM concentration of AZD6738 showed that the rate of formation was linear, with protein and time up to 0.5 mg/ml and 30 minutes, respectively. These conditions were used in the enzyme kinetic study over an AZD6738 concentration range of 0.5–200 μM (Fig. 3).

The deimination showed saturable kinetics with a K_m value of 137 μM and a V_{max} value of 18 pmol/min/mg protein. This provides further support to the deimination being an enzymatic rather than a chemical process.

Recombinant P450 Studies. The rate of metabolism was studied in a panel of 11 recombinant members of the P450 family (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 CYP2J2, CYP3A4, and CYP3A5) at an AZD6738 concentration of 1 μM (Fig. 4A).

Of the enzymes studied, only CYP3A4 and CYP3A5 produced CLint values of 0.177 and 0.04 μl/min/pmol P450, respectively. However, only AZ8982 was detected being formed by CYP2C8 and CYP2J2 at rates of 0.74 and 0.16 pmol/min/pmol P450, respectively. This suggests that although CYP3A has the ability to metabolize AZD6738, only CYP2C8 and CYP2J2 are responsible for forming AZ8982.

In addition, the rate of metabolism was also investigated in recombinant FMO3, FMO5, and NADPH P450-reductase to see whether either of these enzymes was also playing a role. None of these enzymes showed any metabolic activity toward AZD6738 either in terms of disappearance or AZ8982 formation.

AZ8982 was also incubated with the same panel of recombinant members of the P450 family, with CYP3A4 and CYP3A5 again producing the highest CLint values of 2.7 and 0.5 μl/min/pmol P450, respectively (Fig. 4B). These were followed by CYP2J2 and CYP2C8 with CLint values of 0.4 and 0.05 μl/min/pmol P450, respectively. However, in this case the enzymes CYP2J2, CYP2C8, CYP3A4, and CYP3A5 were also responsible for producing sulfone, with rates of 0.34, 0.03, 0.17, and 0.16 pmol/min/pmol P450, respectively, indicating a stronger relationship between the CLint values and the formation of sulfone than was seen for the initial deimination.

Hence, the recombinant enzymes confirm the deimination of AZD6738 as an oxidative process that is mediated by CYP2C8 and CYP2J2. In addition, the subsequent oxidation to the sulfone (AZ0002) is catalyzed by CYP2J2, CYP2C8, CYP3A4, and CYP3A5.

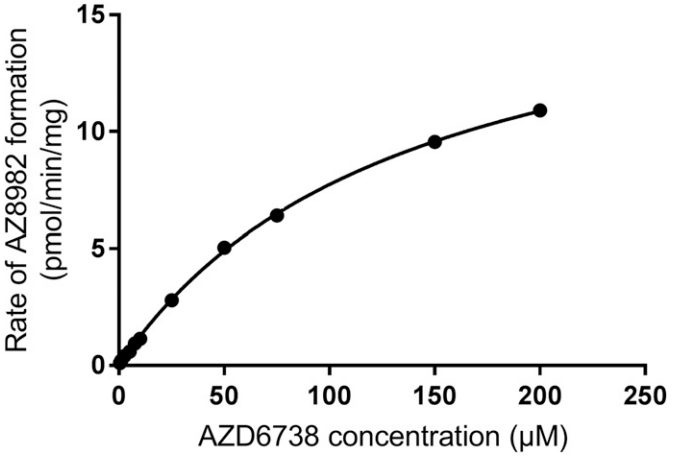

Fig. 3. Saturation kinetics for the formation of AZ8982 from AZD6738.

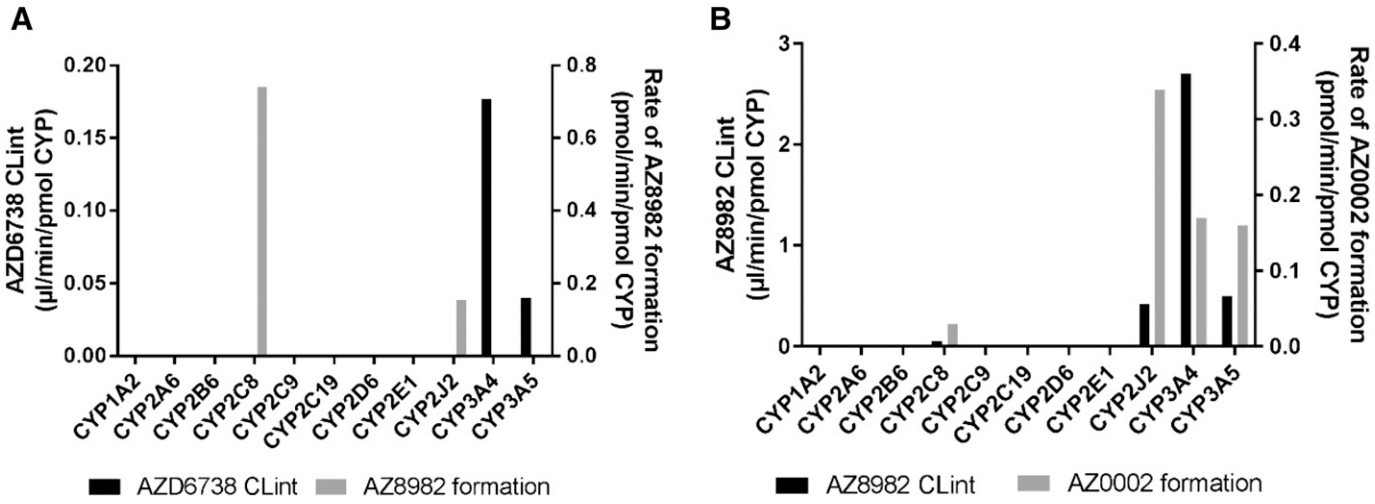

Fig. 4. CLint of AZD6738 compared with AZ8982 formation (A) and CLint and AZ8982 compared with AZ0002 formation (B) in a panel of 11 recombinant members of the P450 family.

Silensomes Studies. To elucidate the enzymes involved in the deimination of AZD6738, the formation of AZ8982 was studied in CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Silensomes (Fig. 5). Given the high K_m values previously determined, these studies were conducted at 25 μM AZD6738.

Silensomes are commercially available HLMs in which specific members of the P450 family have been inhibited by selective mechanism-based inhibitors. Excess inhibitor is then removed during the preparation process, yielding microsomes chemically knocked out for a specific P450 family member (Parmentier et al., 2017).

When AZD6738 was incubated at a concentration of 25 μM, there was no significant difference in the rate of formation of AZ8982 in CYP1A2, CYP2C9, CYP2C19, and CYP2D6 Silensomes compared with the activity seen in the control incubations. However, in CYP2C8 Silensomes the rate was reduced to 5% of the control value, and in CYP3A4 Silensomes the rate of formation of AZ8982 increased by 58% (Fig. 5).

The data from the previous studies in HLMs and recombinant P450 family members suggest that the apparent increase in the rate of formation of AZ8982 formation in CYP3A4 Silensomes was due, in fact, to inhibition of the subsequent metabolic step (the formation of AZ0002) causing a buildup of the sulfoxide. This supports the hypothesis that deimination is the initial metabolic step, which is followed by oxidation of the sulfone. This was confirmed by measuring the CLint value for AZ8982 in Silensomes CYP3A4 and CYP2C8, showing a reduction of 97% and 10%, respectively (data not shown).

Correlation Analysis. The rate of formation of AZ8982 from AZD6738 at 25 μM was studied in set of seven HLM samples from individual samples along with the pooled HLM sample used in the previous studies. In addition, marker enzyme activities for CYP1A2 (phenacetine *O*-de-ethylation), CYP2C8 (amidioquine *N*-de-ethylation), CYP2C9 (diclofenac 4′-hydroxylation), CYP2C19 (*S*-mephenytoin 4-hydroxylation), and CYP3A (midazolam 1′-hydroxylation). All of the marker substrates were incubated on or around the K_m value for the specific reaction. A correlation analysis was conducted between the rate of AZ8982 and the marker P450 enzyme activities (Table 2). There was a poor correlation with the majority of the enzyme activities, with the correlation coefficients determined as being less than 0.5.

The most significant correlation (correlation coefficient = 0.73) was seen between the rate of formation of AZ8982 and the CYP2C8-mediated rate of *N*-de-ethylation of amodiaquine (Fig. 6).

This further supports previous data showing that in HLMs the deimination of AZD6738 is an oxidative process mediated primarily by CYP2C8.

Discussion

Reaction phenotyping is now a relatively routine activity conducted during the discovery and development of drugs. It involves a well-defined set of studies, usually including a combination of metabolite formation by recombinant enzymes, inhibition by specific inhibitors, and correlation across different biologic samples that have been characterized for different enzyme activities (Zientek and Youdim, 2015). In the context of this study, reaction phenotyping has been used to understand the deimination of AZD6738.

This study has investigated the deimination of AZD6738 to its sulfoxide metabolite (AZ8982). It has shown that this is an oxidative process mediated by P450. After this reaction, phenotyping studies including recombinant enzymes, inhibitor (Silensomes), and correlation studies identified CYP2C8 as the major P450 family member involved in this biotransformation. The sulfone metabolite AZ0002 was also

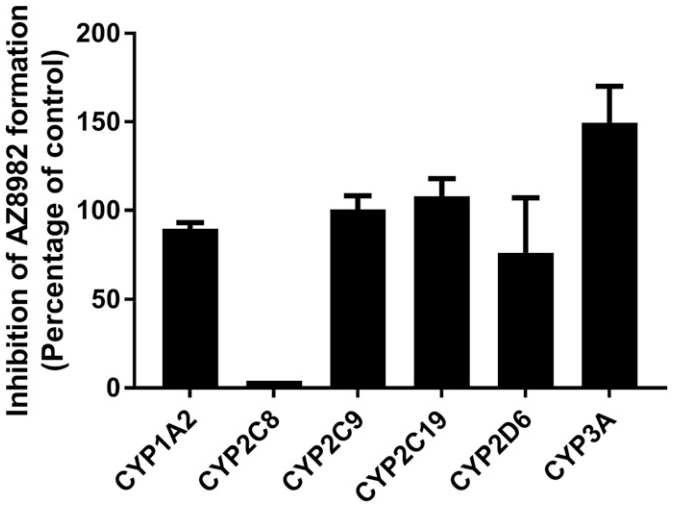

Fig. 5. Formation of AZ8982 from AZD6738 in CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Silensomes.

TABLE 2

Correlation between the rate of deimination of AZD6738 with P450 marker activities across a panel of HLMs from different donors

All activities are reported as picomolar per minute per milligram protein values.

	Rate of Formation of AZ8982	CYP1A2 Activity	CYP2C8 Activity	CYP2C9 Activity	CYP2C19 Activity	CYP2D6 Activity	CYP3A Activity
Rate of Formation of AZ8982	1.00						
CYP1A2 Activity	0.03	1.00					
CYP2C8 Activity	0.76	0.28	1.00				
CYP2C9 Activity	0.41	0.43	0.39	1.00			
CYP2C19 Activity	0.14	0.31	0.21	0.10	1.00		
CYP2D6 Activity	−0.17	−0.24	0.09	0.28	−0.30	1.00	
CYP3A Activity	−0.35	0.65	−0.16	−0.16	−0.03	−0.57	1.00

observed in these studies, but the data suggest that this is a subsequent reaction after the deimination and is mediated by CYP3A.

CYP2J2 was also shown to have a role in the deimination of AZD6738. However, there is a relatively low level of expression of this enzyme in human liver (at around 1.2 pmol/mg protein), which equates to 0.3% of the total hepatic P450 content (Achour et al., 2014). When compared with CYP2C8, which has an expression level of around 22 pmol/mg, which in turn equates to around 5% total hepatic P450 content, it is clear that CYP2J2 would be a minor contributor to the hepatic deimination of AZD6738.

Interestingly, CYP3A did not play a significant role in the deimination of AZD6738. The CLint data from recombinant members of the P450 family show that there was metabolism of AZD6738 by CYP3A but that this did not include the formation of the sulfoxide. This finding was supported by the Silensomes and correlation data. However, CYP3A was the major enzyme involved in the formation of the sulfone (AZ0002). Based on the data from these studies, it is possible to suggest that this is a subsequent step to the initial deimination rather than being formed directly from AZD6738.

CYP2C8 is increasingly recognized as a key human drug-metabolizing enzyme. AZD6738 can now be added to the increasing list of CYP2C8 substrates, which include compounds such as cerivastatin, montelukast, and repaglinide (Backman et al., 2016). In addition, like AZD6738, there are a number of anticancer agents on the substrate list, including paclitaxel, imatinib, and tozasertib. Unlike CYP2C9 and CYP2D6, CYP2C8 does not appear to have a characteristic substrate recognition motif such as an acidic or basic function, although pharmacophore models for CYP2C8 have been proposed that involve a combination of hydrophobic and polar functions at fixed distances from the site of metabolism (Melet et al., 2004). Although AZD6738 may have many of the structural features that align with this type of pharmacophore model, it is difficult to rationalize why the sulfoxide should not be metabolized by the same enzyme but is preferentially metabolized by CYP3A.

There does seem to be a significant overlap between substrates of CYP2C8 and CYP3A (Backman et al., 2016). However, X-rays of crystal structures have revealed that the active sites have a similar size 1438 $Å^3$ for CYP2C8 (Schoch et al., 2004) and 1386 $Å^3$ for CYP3A (Yano et al., 2004) but have different shapes. The active site of CYP3A is generally thought of being a large open space, whereas the CYP2C8 active site has been described as being T or V shaped (Backman et al., 2016). However, these high-level structural differences do not explain why CYP2C8 is able to mediate the deimination of AZD6738 when CYP3A4 cannot, because it is likely that AZD6738 will fit into both of these active sites. In the absence of co–crystal structures of AZD6738 with CYP2C8 and CYP3A4, it is not possible to shed any further light on the differences observed with the selectivity of the deimination reaction.

Having identified CYP2C8 as the major enzyme involved in the deimination of AZD6738, the question becomes, what is the mechanism for this reaction? Nominally going from a sulfoximine to a sulfoxide is a reductive reaction, but the data detailed here show it to actually be an oxidation. This is a kin to the well-described deamination reaction mediated by P450 family members seen with compounds such as diltiazem (Nakamura et al., 1990). However, the mechanism for this reaction is generally described as proceeding via oxidation of the alpha carbon atom adjacent to the nitrogen. In the case of AZD6738, there is no carbon atom adjacent to the imine nitrogen, so this mechanism is unlikely. One other potential mechanism would be similar to the proposed mechanism for N-oxidation (Guengerich, 2001), but in this case after the initial oxidation of the imine nitrogen there is a potential for subsequent oxidation and liberation of nitrous acid to yield the sulfoxide (Fig. 7).

In summary, these in vitro studies show that AZD6738 undergoes oxidative deimination to the corresponding sulfoxide (AZ8982) mediated principally by CYP2C8. AZ8982 undergoes subsequent CYP3A-mediated oxidation to the sulfone (AZ0002). This represents one of the first studies of the metabolism of the sulfoximine moiety.

Authorship Contributions

Participated in research design: Jones, Markandu, and Scarfe.

Conducted experiments: Markandu.

Performed data analysis: Jones and Markandu.

Wrote or contributed to the writing of the manuscript: Jones, Markandu, Gu, and Scarfe.

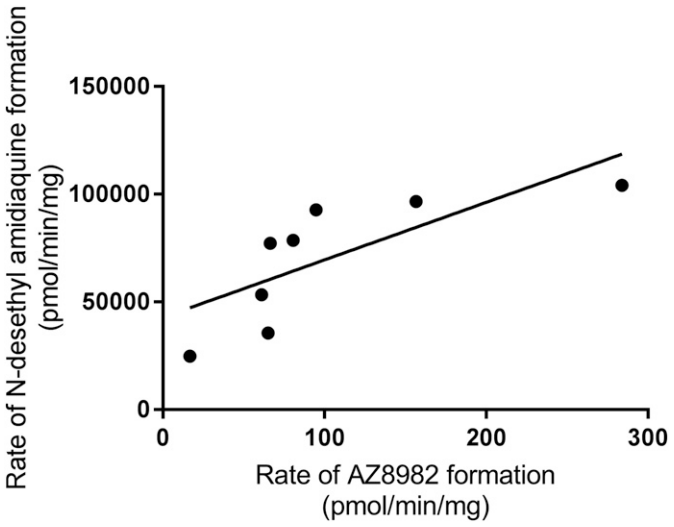

Fig. 6. Correlation between rate of AZ8982 formation and CYP2C8 activity as measured by amadiaquine *N*-de-ethylase activity across a panel of HLM samples from different donors.

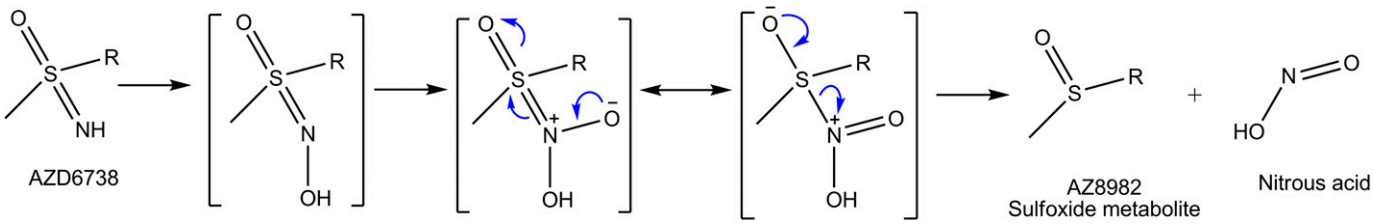

Fig. 7. Proposed mechanism for the oxidative deimination of AZD6738.

References

Achour B, Barber J, and Rostami-Hodjegan A (2014) Expression of hepatic drug-metabolizing cytochrome p450 enzymes and their intercorrelations: a meta-analysis. *Drug Metab Dispos* **42**: 1349–1356.

Ahluwalia GS, Grem JL, Hao Z, and Cooney DA (1990) Metabolism and action of amino acid analog anti-cancer agents. *Pharmacol Ther* **46**:243–271.

Argikar UA, Potter PM, Hutzler JM, and Marathe PH (2016) Challenges and opportunities with non-CYP enzymes aldehyde oxidase, carboxylesterase, and UDP-glucuronosyltransferase: focus on reaction phenotyping and prediction of human clearance. *AAPS J* **18**:1391–1405.

Backman JT, Filppula AM, Niemi M, and Neuvonen PJ (2016) Role of cytochrome P450 2C8 in drug metabolism and interactions. *Pharmacol Rev* **68**:168–241.

Bahleda R, Grilley-Olson JE, Govindan R, Barlesi F, Greillier L, Perol M, Ray-Coquard I, Strumberg D, Schultheis B, Dy GK, et al. (2017) Phase I dose-escalation studies of roniciclib, a pan-cyclin-dependent kinase inhibitor, in advanced malignancies. *Br J Cancer* **116**:1505–1512.

Cimprich KA and Cortez D (2008) ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* **9**:616–627.

Foote KM, Lau A, and Nissink JW (2015) Drugging ATR: progress in the development of specific inhibitors for the treatment of cancer. *Future Med Chem* **7**:873–891.

Glasauer A and Chandel NS (2014) Targeting antioxidants for cancer therapy. *Biochem Pharmacol* **92**:90–101.

Guengerich FP (2001) Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* **14**:611–650.

Guichard SM, Brown E, Odedra R, Hughes A, Heathcote D, Barnes J, Lau A, Powell S, Jones CD, Nissink W, et al. (2013) Abstract 3343: the pre-clinical in vitro and in vivo activity of AZD6738: a potent and selective inhibitor of ATR kinase. *Cancer Res* **73**:3343.

Jones CD, Blades K, Foote KM, Guichard SM, Jewsbury PJ, McGuire T, Nissink JW, Odedra R, Tam K, Thommes P, et al. (2013) Abstract 2348: discovery of AZD6738, a potent and selective inhibitor with the potential to test the clinical efficacy of ATR kinase inhibition in cancer patients. *Cancer Res* **73**:2348.

Melet A, Marques-Soares C, Schoch GA, Macherey AC, Jaouen M, Dansette PM, Sari MA, Johnson EF, and Mansuy D (2004) Analysis of human cytochrome P450 2C8 substrate specificity using a substrate pharmacophore and site-directed mutants. *Biochemistry* **43**:15379–15392.

Nakamura S, Ito Y, Fukushima T, Sugawara Y, and Ohashi M (1990) Metabolism of diltiazem. III. Oxidative deamination of diltiazem in rat liver microsomes. *J Pharmacobiodyn* **13**:612–621.

Parmentier Y, Pothier C, Delmas A, Caradec F, Trancart MM, Guillet F, Bouaita B, Chesne C, Brian Houston J, and Walther B (2017) Direct and quantitative evaluation of the human CYP3A4 contribution (fm) to drug clearance using the in vitro SILENSOMES model. *Xenobiotica* **47**:562–575.

Schoch GA, Yano JK, Wester MR, Griffin KJ, Stout CD, and Johnson EF (2004) Structure of human microsomal cytochrome P450 2C8. Evidence for a peripheral fatty acid binding site. *J Biol Chem* **279**:9497–9503.

Sirvent JA and Lucking U (2017) Novel Pieces for the Emerging Picture of Sulfoximines in Drug Discovery: Synthesis and Evaluation of Sulfoximine Analogues of Marketed Drugs and Advanced Clinical Candidates. *Chem Med Chem* **12**:487–501.

Weber AM and Ryan AJ (2015) ATM and ATR as therapeutic targets in cancer. *Pharmacol Ther* **149**:124–138.

Yano JK, Wester MR, Schoch GA, Griffin KJ, Stout CD, and Johnson EF (2004) The structure of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-A resolution. *J Biol Chem* **279**:38091–38094.

Zientek MA and Youdim K (2015) Reaction phenotyping: advances in the experimental strategies used to characterize the contribution of drug-metabolizing enzymes. *Drug Metab Dispos* **43**: 163–181.

Address correspondence to: Barry C. Jones, Hodgkin Building c/o B310, Cambridge Science Park, Milton Road, Cambridge CB4 0WG, Cambridgeshire, UK. E-mail: barry.jones1@astrazeneca.com