

DMD #084103

**Characterization of the in vitro inhibitory potential of the
oligonucleotide imetelstat on human cytochrome P450 enzymes with
predictions of in vivo drug-drug interactions**

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Running Title: P450 based DDI potential of the oligonucleotide imetelstat

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Document Summary:

Number of Text Pages	16
Number of Tables	2
Number of Figures	2
Number of References	28
Number of Words in the Abstract	243
Number of Words in the Introduction	590
Number of Words in the Discussion	1285

Abbreviations used are: ASO, antisense oligonucleotide; AUC, area under the curve; CHH, cryopreserved human hepatocytes; DDI, drug-drug interaction; EMA, European Medicines Agency; ESI; electrospray ionization; FDA, U.S. Food and Drug Administration; f_m , fractional metabolism by a clearance pathway; HLM, human liver microsomes; IC_{50} , inhibitor concentration that causes 50% inhibition; KHB; Krebs-Henseleit buffer; LC-MS/MS, liquid chromatography/tandem mass spectrometry.

ABSTRACT

Imetelstat, a 13-base oligonucleotide (5'-TAGGGTTAGACAA-3') is a potent, investigational telomerase inhibitor in clinical development for the treatment of hematologic myeloid malignancies. Modifications to imetelstat oligonucleotide chemistry include a N3'-P5' thiophosphoramidate backbone linkage to improve biological stability and the addition of a palmitoyl tail at the 5'-position to enhance cellular membrane permeability. Other oligonucleotides have been previously shown to have *in vitro* test-system dependent outcomes where potent P450 inhibition in human liver microsomes (HLM) is observed but such inhibition is not observed in cryopreserved human hepatocytes (CHH). Outcomes in CHH are consistent with clinical reports where no interactions are observed. In the present study, imetelstat was evaluated for *in vitro* inhibition of eight P450 enzymes, namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 in CHH (0.5 million cells/mL). Assays were performed using validated conditions, including short substrate times (10 min) and at the approximate substrate K_m concentration. Imetelstat was found to have little to no inhibition of all P450 isoforms evaluated, with IC_{50} values $>100 \mu\text{M}$. Maximum percent inhibition values for each P450 isoform at $100 \mu\text{M}$ imetelstat were $<20\%$ except for CYP2C8 activity which was inhibited by 49%. Using a static mechanistic model, the predicted change in AUC of a victim drug co-administered with imetelstat was 1.04-fold, predicting no relevant clinical interaction. Overall, the results from this *in vitro* study suggest that clinical use of imetelstat is unlikely to affect the pharmacokinetics of concomitant therapies that undergo P450 mediated metabolism.

INTRODUCTION

Oligonucleotide based therapeutics are an attractive area for pharmaceutical development due to the ability to provide target specificity based on base-pair sequence, especially in the areas of cancer treatment, genetic disorders, infectious diseases, and metabolic disorders (Lundin et al., 2015; Sharma and Watts, 2015; Aartsma-Rus, 2016). Usually these molecules function by having a base pair sequence anti-sense complementary to a target mRNA sequence, leading to down regulation of mRNA translation and protein expression, and are thus also known as anti-sense oligonucleotides (ASOs). Chemical modifications of oligonucleotides have been adopted as strategies to enhance target interaction and biological stability, yielding a general improvement in pharmacokinetic and pharmacodynamic properties of these therapeutics. This includes sulfurization of the phosphodiester bond, methoxy or methoxyethyl group addition to sugar moieties, locked/unlocked nucleic acids, and inter-nucleotide linkage modifications to form peptide nucleic acids (Prakash, 2011; Deleavey and Damha, 2012; Sharma and Watts, 2015; Chen et al., 2018). Among the many oligonucleotide biomolecules investigated thus far, only fomivirsen, pegaptanib, mipomersen, eteplirsen, nusinersen and defibrotide sodium have received FDA approval (Stein and Castanotto, 2017).

Imetelstat (13-mer oligonucleotide N3'-P5' thio-phosphoramidate with a covalently-linked C16 [palmitoyl] lipid moiety at the 5' end), is in clinical development as a treatment option for hematologic myeloid malignancies (Roth et al., 2010; Chiappori et al., 2015; Baerlocher et al., 2015; Tefferi et al., 2015; Dillen et al., 2017). Imetelstat is a high-affinity, active site telomerase inhibitor with specificity for the RNA template region of telomerase, directly competing with telomere binding. Therefore, its mode of action is similar to a conventional small molecule competitive enzyme inhibitor rather than like an ASO (knockdown of target) (Herbert et al., 2005). Activation of telomerase is thought to be important for tumor progression by enabling cancer cells to maintain short telomeres, gain immortality, and evade apoptosis (Lichtsteiner et al., 1999). The

mechanism of action by imetelstat results in potent inhibition of telomerase and leads to progressive telomere shortening (Herbert et al., 2005; Marian et al., 2010). Structurally (see Figure 1), the thio-phosphoramidate linkage within imetelstat confers high resistance to nuclease digestion in blood and tissues, while the properties of the 5'-palmitoyl chain result in improved cellular permeability and consequently strong tissue penetration and retention (Herbert et al., 2005).

The potential for oligonucleotide based molecules to act as perpetrators of drug-drug interactions (DDIs) has not been reported extensively in the literature. However, of the clinical interaction studies conducted with victim drugs subject to clearance by P450, glucuronidation, renal elimination, or nucleoside kinases, none has reported a clinically relevant DDI by therapeutic oligonucleotides (Adjei et al., 2003; Villalona-Calero et al., 2004; Geary et al., 2006; Yu et al., 2009; Li et al., 2014). Industry regulatory guidances from the FDA (2017 draft - <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965.pdf>) and the EMA (2013 final - http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf) recommend the conduct of *in vitro* P450 inhibition studies for small molecule drugs in appropriate test systems (such as human liver microsomes or cryopreserved human hepatocytes) to ascertain the perpetrator DDI potential of a drug. Recent studies on P450 inhibition *in vitro* suggest that cryopreserved human hepatocytes (CHH) are a more clinically relevant test system for the prediction of DDIs for oligonucleotides, and that studies conducted in human liver microsomes may result in artefactual results (Kazmi et al., 2018). This is supported by another recent *in vitro* report where no P450 inhibition by oligonucleotides was observed in CHH (Shemesh et al., 2017). In the present study, the *in vitro* P450 inhibitory potential of imetelstat was evaluated in CHHs to determine the risk for P450 mediated DDIs with concomitantly administered medications.

MATERIALS AND METHODS

Chemicals and Reagents

Imetelstat, a 13-base oligonucleotide (5'-TAGGGTTAGACAA-3') N3'-P5' thio-phosphoramidate containing a palmitoyl tail at the 5'-position (5'-palmitoyl-TAGGGTTAGACAA-NH₂-3') was provided by Geron Corporation (Menlo Park, CA). Krebs-Henseleit buffer (KHB), calcium chloride, phenacetin, bupropion, tolbutamide, dextromethorphan, chlorzoxazone, midazolam, α -naphthoflavone, ticlopidine, sulfaphenazole, quinidine, 4-methylpyrazole, ketoconazole, amodiaquine, montelukast, and acetaminophen were all purchased from Sigma-Aldrich (St. Louis, MO). Sodium bicarbonate and HEPES were purchased from Life Technologies (Carlsbad, CA). *N*-3-benzylphenobarbital was obtained internally from Janssen Research & Development (Beerse, Belgium); *S*-(+)-Mephénytoin was purchased from Corning Life Sciences, USA; metabolite reference standards including hydroxytolbutamide, (+/-)-4'-hydroxymephénytoin, dextrorphan, 6-hydroxychlorzoxazone, 1'-hydroxymidazolam, hydroxybupropion and *N*-desethylamodiaquine were purchased from Ultrafine Chemicals and BD Gentest, both formerly part of BD Biosciences and now part of Corning Discovery Labware, Inc. (Woburn, MA). Hepatocyte Thawing Media was purchased from Bioreclamation IVT (Baltimore, MD). The Strata Impact protein precipitation 96-well plates were purchased from Phenomenex Corporation (Torrance, CA, USA). All other reagents and solvents were of HPLC grade or equivalent analytical standards.

Test systems

Pooled cryopreserved human hepatocytes, lot PDI (CHH, n = 20, mixed gender) were purchased from Bioreclamation IVT (Baltimore, MD). Hepatocyte incubations were performed in oxygen sparged KHB supplemented with calcium chloride, sodium bicarbonate and HEPES. Buffer pH was adjusted to 7.4 ± 0.1 .

In vitro P450 inhibition

Imetelstat (0.1-100 μM) was evaluated for its potential to reversibly inhibit eight P450 enzymes, namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 in CHH at the approximate substrate K_m concentration (determined in house with the same lot of CHH) as shown in Table 1. Triplicate incubations for each concentration were conducted with an automated liquid handling system (Biomek, Beckman Coulter, Indianapolis, IN) in 100 μL mixtures at 0.5 million cells/mL in KHB. After serial dilution in KHB, imetelstat or experimental controls (single concentrations) and the probe substrates were aliquoted equally (10 μL each) into the incubation vessels. The final organic content was $\leq 0.05\%$ for imetelstat and $\leq 0.15\%$ for experimental controls (with the exception of $\leq 0.85\%$ for montelukast) in each incubated sample (considering the substrate and the inhibitor). Reactions were initiated with the addition of 80 μL CHH to the incubation vessels and incubations were conducted at 37°C with 95% humidity, 5/95% CO_2/air , and 350 rpm shaking for 10 min. Reactions were quenched after 10 min by the addition of 200 μL of acetonitrile containing an appropriate mixture of internal standards. After incubation, the samples were transferred to a 2 mL Strata Impact protein precipitation 96 well plate containing 300 μL acetonitrile. The samples were filtered on a vacuum manifold, the filtrate was evaporated to dryness under nitrogen and then reconstituted in 250 μL mobile phase (1:1, methanol:water containing 0.1% acetic acid) for further LC-MS/MS analysis using a cocktail method (see Table 1). Early screening studies for P450 inhibition by imetelstat in HLM were conducted by In Vitro Technologies Inc. (Baltimore, MD) and Cerep (Redmond, WA) as described in Supplemental Table 1.

Analytical methods

Samples were analyzed by liquid chromatography /tandem mass spectrometry (LC/MS-MS) on a Shimadzu Nexera liquid chromatography system (binary LC-30AD pumps with SIL-30ACMP autosampler (Shimadzu Scientific Instruments, Columbia, MD, USA), coupled to a SCIEX API 4000 triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA, USA) operated in multiple reaction monitoring (MRM) and electrospray ionization (ESI) scan modes. For analysis of metabolites, a gradient elution method comprising 0.1% acetic acid in water (A) and methanol with 0.1% acetic acid (B) ramping from 6 to 90% over 9 minutes (vs. 4 min for 6-hydroxychlorzoxazone, negative ESI mode) was applied to a Zorbax Eclipse Plus Phenyl Hexyl column (2.1 x 100 mm, 1.8 μ m; Agilent Technologies, Santa Clara, CA) and column temperature was maintained at 50°C.

Data analyses

The acquired data were processed using Analyst® version 1.6.2 (AB SCIEX, Foster City, CA) and the area ratios of metabolite and internal standard peaks were exported to Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Nonlinear regression and IC₅₀ curve fitting analysis were performed using SigmaPlot, version 12.5 (Systat Software, Inc., Chicago, IL). Inhibition was measured as the percent reduction of probe substrate metabolite formed in the presence of imetelstat compared to the amount formed in the absence of test compound (maximal activity). Area ratios (AR) of the metabolite peak to the internal standard peak were used in the calculations, using equation (1):

$$\% \text{ Inhibition} = 100 \times [(AR_{(\text{No Inhibitor})} - AR_{(+\text{imetelstat})}) / AR_{(\text{No Inhibitor})}] \quad (1)$$

Residual activity (i.e. maximal activity - % inhibition) was plotted as a function of inhibitor concentration (numerical data in Supplemental Table 2). Prediction of hepatocyte fraction unbound ($f_{u\text{inc}}$) was determined by calculation of a predicted imetelstat logP with MarvinSketch version 16.1.11 (ChemAxon, Cambridge MA) followed by application of the $f_{u\text{inc}}$ equation described previously by Kilford et., al (2008).

Imetelstat inhibition data were analyzed with basic and mechanistic static models for the assessment of DDI risk as described by the current FDA and EMA guidance documents. The static mechanistic model describing hepatic only DDI risk is shown in equation (2):

$$\text{AUC ratio} = \left(\frac{1}{[A \times B \times C] \times f_m + (1 - f_m)} \right) \quad (2)$$

Where A = reversible inhibition or $(1/(1+[I_h]/K_i))$; B = Time dependent inhibition (TDI) parameters; and C = induction parameters. Because imetelstat is dosed intravenously, gut parameters were excluded. B was assumed to equal 1 due to a lack of TDI by imetelstat (Supplemental Figure 1). Induction parameters were not considered (i.e. C = 1) per the current FDA and EMA guidance recommendations on assessment of inhibition only DDI potential. I_h = hepatic inlet concentration = fraction unbound in plasma ($f_{u_{\text{plasma}}}$) \times $[C_{\text{max}} + \text{fraction absorbed } (F_a) \times \text{absorption rate constant } (k_a) \times \text{dose/hepatic blood flow } (Q_h)/\text{blood to plasma ratio } (R_b)]$.

RESULTS

Inhibition of P450 enzymes by imetelstat in cryopreserved human hepatocytes. Imetelstat (0.1-100 μM) was evaluated as a potential direct inhibitor of P450 isoforms in CHH (0.5 million cells/mL) at the approximate substrate K_m concentration (see Table 1) as described in *Materials and Methods*. As shown in Figure 2 and Table 2, imetelstat had little to no inhibitory effect on CYP1A2 (phenacetin), CYP2B6 (bupropion), CYP2C8 (amodiaquine), CYP2C9 (tolbutamide), CYP2C19 (S-mephenytoin), CYP2D6 (dextromethorphan), CYP2E1 (chlorzoxazone) and CYP3A4 (midazolam) with IC_{50} values all $>100 \mu\text{M}$. The maximum percent inhibition at the highest concentration of imetelstat tested (100 μM) was less than 20% for all P450 isoforms, except for CYP2C8 where the maximum inhibition was 49%. However, a modest inhibitory concentration dependency for CYP2C8 was observed, with inhibition plateaued between 30-100 μM imetelstat. In contrast, all positive control inhibitors showed $>80\%$ inhibition of their respective P450 isoforms as expected (see Table 2). Furthermore, a lack of CYP2C8 TDI by imetelstat was observed with an IC_{50} shift assay (Supplemental Figure 1).

DISCUSSION

For small molecule drugs, regulatory agencies such as the FDA and EMA recommend in vitro evaluation of the drug-drug interaction (DDI) potential of a new molecular entity as part of the submission package towards drug approval. This is typically done through in vitro assessment of the inhibition of drug metabolizing enzymes, namely P450 enzymes, in validated test-systems such as human liver microsomes (HLM) or cryopreserved human hepatocytes (CHH). In the case of oligonucleotide drugs, although these biomolecules as a class differ in structure and composition from conventional small molecule drugs, assessment of the DDI potential of oligonucleotides is typically evaluated in a manner similar to the FDA and EMA DDI study recommendations outlined for small molecule drugs. Thus, in the present study the in vitro potential for imetelstat to inhibit P450 enzymes, namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 was evaluated in CHH.

As shown in Figure 2 and Table 2, imetelstat was found not to be an in vitro inhibitor of any P450 enzymes tested in CHH with IC_{50} values greater than 100 μ M. Furthermore, the maximum percent inhibition at highest imetelstat concentration (100 μ M) was minimal (<20%) for all P450 isoforms, except for CYP2C8 activity (49% inhibition), where modest concentration dependency was observed. However, it is unclear why the inhibition of CYP2C8 plateaued between 30 and 100 μ M (45-49% inhibition), as this is typically observed when inhibitor solubility is an issue; a phenomenon not observed with imetelstat (high aqueous solubility). Overall, these findings are consistent with other in vitro reports that oligonucleotides as a class do not cause significant inhibition of P450 enzymes in CHH (Shemesh et al., 2017; Kazmi et al., 2018). The recent report by Kazmi and colleagues (2018) suggests that oligonucleotide molecules as a class may cause differential test-system dependent inhibition in HLM and CHH based on the chemical properties of the oligonucleotide backbone (phosphodiester versus phosphorothioate linkage), and that inhibition profiles for oligonucleotides in CHH are more clinically relevant. In the case of imetelstat,

a thio-phosphoramidate linkage is present (see Figure 1) along with a 5'-palmitoyl chain for the improvement of biological stability and tissue uptake (Herbert et al., 2005). Liver uptake of imetelstat has been previously observed in mice (Herbert et al., 2005), consistent with in house preclinical toxicology studies where liver distribution of imetelstat was seen in rodents and primates, confined largely to Kupffer cells (data not shown). This accumulation into tissue macrophages (often observed as basophilic granules) has been shown to be a class effect of oligonucleotides, especially with high doses (Frazier, 2015). In screening studies, potent P450 inhibition by imetelstat was observed in HLM for CYP2C8 (IC₅₀ value of 0.7 μM) with IC₅₀ values for other P450 enzymes spanning 1.8-26 μM (see Supplemental Table 1). The P450 inhibition observed in HLM, particularly of CYP2C8, was completely attenuated in the present study conducted with CHH suggesting test-system dependent inhibition, possibly due to the thio-phosphoramidate linkage of imetelstat.

With respect to predictions of a clinical DDI, the static basic model approach, as recommended by the EMA and FDA, was first utilized where $R_1 = 1 + I_{\max,u}/K_i$, with R_1 representing the predicted AUC ratio in the presence or absence of inhibitor, $I_{\max,u}$ representing maximal free plasma concentration of the inhibitor, and K_i representing the unbound *in vitro* inhibition constant. Imetelstat C_{\max} concentration with a 2 hr intravenous infusion of 9.4 mg/kg dose was approximately 30 μM (136 μg/mL). The *in vitro* K_i value for P450 inhibition can be predicted reliably from the IC₅₀ value by using the equation $IC_{50}/2 = K_i$ (Haupt et al., 2015). Non-specific binding of imetelstat to CHH was expected to be negligible ($f_{u,inc} = 1$) based on physicochemical properties (hydrophilicity [predicted logP = -3.19] and charge status), and application of the predicted hepatocyte binding equation described by Kilford et al, (2008). In the case of CYP2C8 (the P450 enzyme with the greatest maximal inhibition by imetelstat), the K_i was estimated to be 50 μM based on an approximate IC₅₀ value of 100 μM in CHH. Considering the plasma protein binding of imetelstat (at least 92.7%; Janssen internal data), the R_1 value was calculated as 1.04 which

is above the EMA and FDA cut-off criteria of $R_1 \geq 1.02$. The basic static models recommended by the EMA and FDA are designed to be stringent in nature by including a 50-fold safety margin for I/K_i ($I = 1$, $K_i = 50$; or $I/K_i = 0.02$). Should an assessment with the basic static model fail to pass DDI cut-off criteria, the EMA and FDA recommend assessment of DDI prediction with a static mechanistic model or a dynamic model, which incorporate much more detailed drug disposition parameters than the basic static model (Fahmi et al., 2009). For the static mechanistic model, these parameters include the effect of hepatic reversible inhibition, time-dependent inhibition (TDI), enzyme induction, and hepatic inlet concentration of a perpetrator drug; as well as fractional metabolism (f_m) of a victim drug. However, the EMA and FDA recommend, in their most recent DDI guidelines, that inhibitory potential and induction potential with the static mechanistic model be considered separately and not simultaneously as was originally proposed by Fahmi and colleagues (2009). Thus, predictions of inhibitory perpetrator DDI potential with this model are to be based on an assessment of direct and time dependent inhibition only (as shown in Equation 2). Furthermore, use of the static mechanistic approach described by the EMA and FDA in their respective DDI guidances, requires an estimate of hepatic inlet concentration (I_h) that is obtained by including absorption, dose, blood flow and blood partitioning parameters in addition to plasma C_{max} and plasma protein binding parameters (Ito et al., 1998). However, in the case of imetelstat, because it is dosed intravenously, oral absorption parameters are not applicable and the hepatic inlet concentration is equivalent to the unbound systemic plasma concentration ($f_{u,plasma} * C_{max} = I_h = 2.19 \mu\text{M}$). Imetelstat is not expected to be a time-dependent inhibitor of CYP2C8 (Supplemental Figure 1), and assuming a worst-case scenario of a victim drug with 100% systemic clearance through one P450 pathway ($f_m = 1$), application of these parameters to the static mechanistic model yields a prediction of 1.04-fold change in the AUC of a victim drug that is a substrate of CYP2C8. This is well below the EMA and FDA AUC ratio cut-off criteria of ≥ 1.25 and indicates that imetelstat would likely not be a perpetrator of a clinical DDI.

The predicted lack of clinical DDI for imetelstat is consistent with the reported lack of clinical interactions with other oligonucleotides. Clinical pharmacokinetic interaction studies with simvastatin (CYP3A4 mediated clearance), warfarin (CYP2C9/3A4/1A2 mediated clearance), rosiglitazone (CYP2C8/2C9 mediated clearance), glipizide (CYP2C9/2C8 mediated clearance), ezetimibe (glucuronidation mediated clearance), metformin (renal transporter mediated clearance), cisplatin (renal and copper transporter mediated clearance) and gemcitabine (nucleoside kinase mediated clearance) have all shown no significant pharmacokinetic interaction with therapeutic oligonucleotides (Adjei et al., 2003; Villalona-Calero et al., 2004; Geary et al., 2006; Yu et al., 2009; Li et al., 2014). Furthermore, an in vivo rat preclinical interaction study with imetelstat co-administered with paclitaxel, which is metabolized by CYP3A1/2 in rat (Vaclavikova et al., 2004), showed no significant pharmacokinetic interaction (Janssen internal data), supporting the lack of reported DDIs by oligonucleotide based therapeutics.

In conclusion, the findings presented in this study indicate that imetelstat is not a significant in vitro inhibitor of P450 enzymes in CHH, and the in vitro to in vivo extrapolation (IVIVE) of DDI potential using the EMA and FDA recommended static mechanistic model also projects imetelstat to not likely be a clinically P450 significant inhibitor, and thus consequently unlikely to affect the pharmacokinetics of concomitant pharmacotherapies that are metabolized by P450s.

ACKNOWLEDGMENTS

Medical writing assistance was provided by Ashwini Patil (Tata Consultancy Services, Mumbai, India) and additional editorial support was provided by Namit Ghildyal, PhD (Janssen Research & Development, LLC). We would like to thank Shannon Dallas, PhD and Avijit Ghosh, PhD for helpful discussions and manuscript review.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Kazmi, Sensenhauser, Greway

Conducted Experiments: Kazmi, Sensenhauser

Performed data analysis: Kazmi, Sensenhauser

Wrote or contributed to the writing of the manuscript: Kazmi, Sensenhauser, Greway

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FIGURE LEGENDS

Fig. 1: Truncated structures of (A) imetelstat, (B) phosphorothioate and (C) phosphodiester oligonucleotide linkages. The thio-phosphoramidate, phosphorothioate and phosphodiester linkages on each respective molecule are highlighted in red.

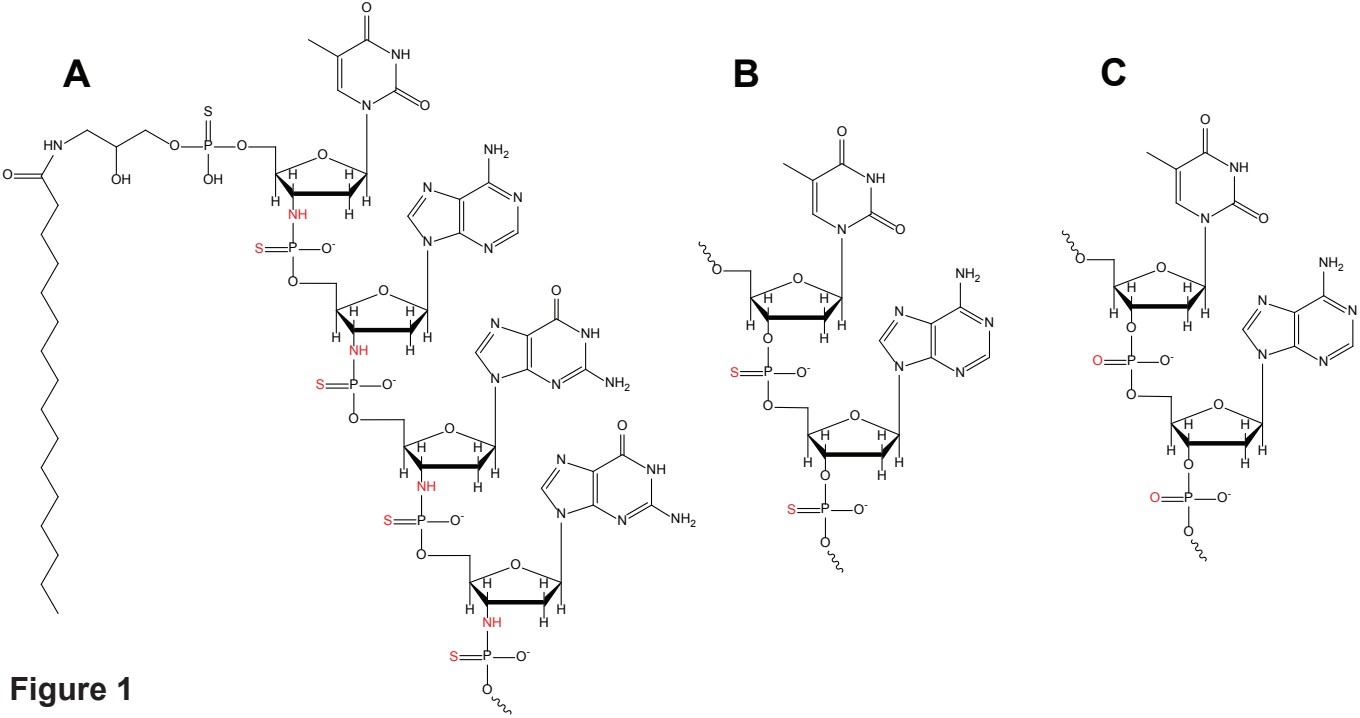
Fig. 2: IC₅₀ plots of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 inhibition by imetelstat in cryopreserved human hepatocytes (CHH). As described in *Materials and Methods* and Table 1, the inhibition of P450 enzymes was assessed at 0.1-100 μM imetelstat (n = 3) in CHH at 0.5 million cells/mL for 10 min followed by LC-MS/MS analysis. Curve fitting was not performed where IC₅₀ values were projected to be > 100 μM.

Table 1: Experimental conditions for the measurement of P450 activity in pooled cryopreserved human hepatocytes (CHH)

Enzyme	Marker activity	Substrate concentration (μM)	CHH (million cells/mL)	Incubation time in (min)	Mass transition monitored (m/z)	Ionization mode	Internal Standard
CYP1A2	Phenacetin O-deethylation	20	0.5	10	152 / 110	ESI+	d ₄ -Acetaminophen
CYP2B6	Bupropion hydroxylation	10	0.5	10	256 / 167	ESI+	d ₆ -Hydroxybupropion
CYP2C8	Amodiaquine N-dealkylation	3	0.5	10	328 / 283	ESI+	d ₃ -N-Desethylamodiaquine
CYP2C9	Tolbutamide hydroxylation	20	0.5	10	287 / 171	ESI+	d ₉ -Hydroxytolbutamide
CYP2C19	S-Mephenytoin 4'-hydroxylation	5	0.5	10	235 / 150	ESI+	d ₃ -4'-Hydroxymephenytoin
CYP2D6	Dextromethorphan O-demethylation	1	0.5	10	258 / 157	ESI+	d ₃ -Dextrorphan
CYP2E1	Chlorzoxazone 6-hydroxylation	45	0.5	10	184 / 120	ESI-	d ₂ -6-hydroxychlorzoxazone
CYP3A4	Midazolam 1'-hydroxylation	2	0.5	10	342 / 324	ESI+	¹³ C ₃ -1'-Hydroxymidazolam

Table 2: IC₅₀ and maximum percent inhibition values for the inhibition of P450 isoforms by imetelstat and positive control inhibitors in pooled cryopreserved human hepatocytes (CHH)

Enzyme	Substrate	IC ₅₀ (μM)	Maximum inhibition (%)	Positive control Inhibitor (concentration)	Positive control inhibition (%)
CYP1A2	Phenacetin	>100	< 10	α-Naphthoflavone (1 μM)	90
CYP2B6	Bupropion	>100	< 10	Ticlopidine (10 μM)	84
CYP2C8	Amodiaquine	>100	49	Montelukast (50 μM)	88
CYP2C9	Tolbutamide	>100	< 10	Sulfaphenazole (5 μM)	99
CYP2C19	S-Mephenytoin	>100	< 10	N-3-Benzylphenobarbital (4 μM)	96
CYP2D6	Dextromethorphan	>100	14	Quinidine (5 μM)	99
CYP2E1	Chlorzoxazone	>100	10	4-Methylpyrazole (10 μM)	92
CYP3A4	Midazolam	>100	18	Ketoconazole (4 μM)	98



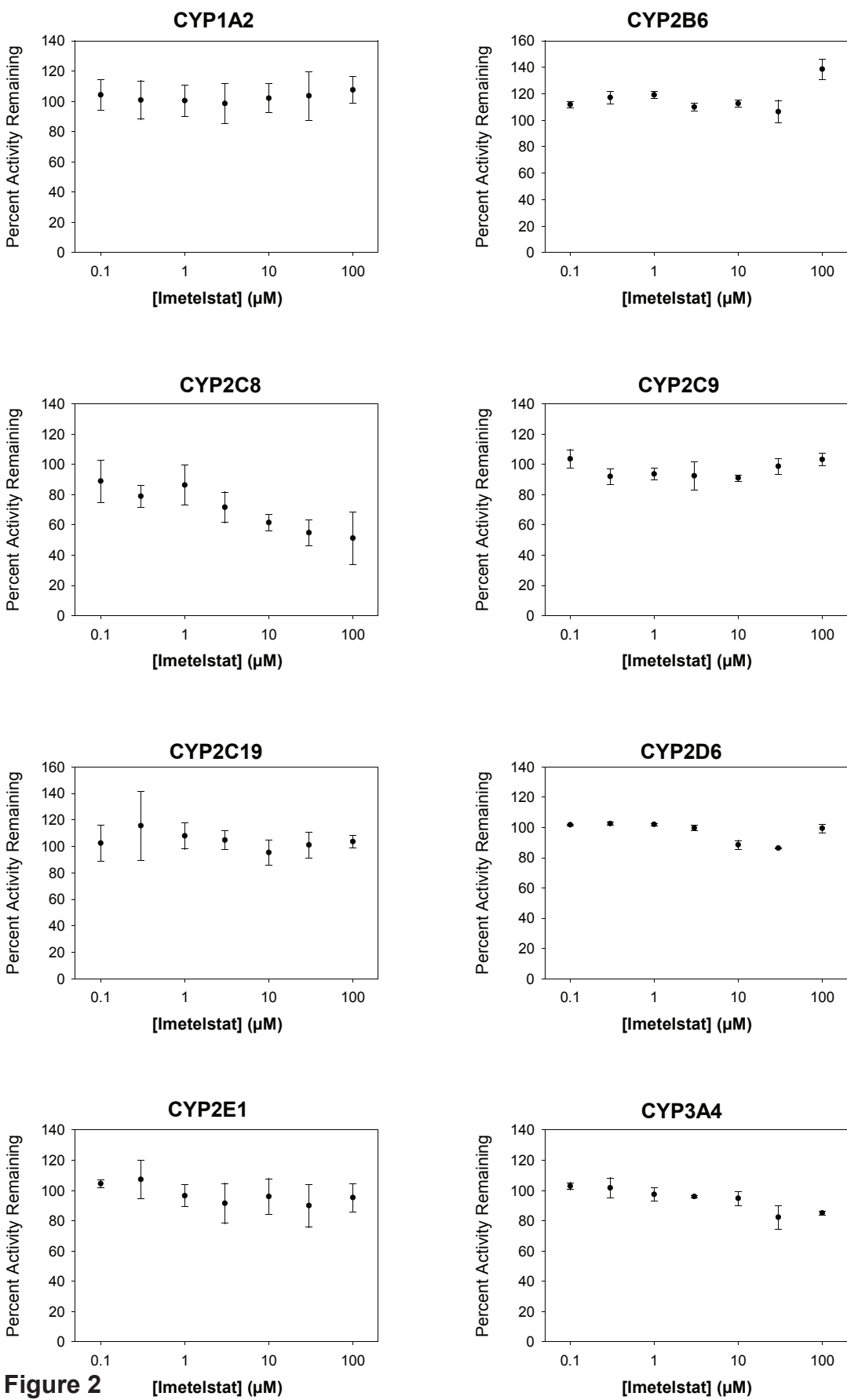


Figure 2