SUPPLEMENTARY INFORMATION

Aim: To evaluate: A) *in vitro* metabolic stability of voriconazole in mouse liver microsomes; B) presence of human CYP2C9, 2C19 and 3A4/5 orthologs in mouse liver microsomes.

Method: Voriconazole at three different concentrations (0.25, 0.5 and 1µM) was incubated with 0.5 mg/mL protein concentration of mouse liver microsomes (Sekisui XenoTech, LLC, Lenexa, Kansas, USA) in presence of 100 mM phosphate buffer pH 7.4 and 1 mM NADPH 0, 5, 15, 30 and 60 min in a water bath (Julabo SW23) at 37 °C and 80 rpm shaking (final organic concentration 0.1% v/v). The reactions were stopped by addition of 600 µL of acetonitrile containing 5 ng/mL alprazolam as internal standard.

Similarly separate incubations of 1 µM voriconazole were performed with 5µM concentration of each CYP2C9, CYP2C19 and CYP3A4/5 selective inhibitor sulfaphenazole, ticlopidine and ketoconazole, respectively.

Similarly probe substrate of CYP2C9- diclofenac, CYP2C19- S-mephenytoin and CYP3A4/5-midazolam were incubated at 1µM concentration to test presence of human CYP orthologs. Testosterone was also incubated at 1µM concentration to test enzyme activity.

All samples were vortexed for 1 min and centrifuged at 3000 rpm for 10 min. The supernatant were transferred to a 96 well collection plate and analyzed by LC-MS/MS method.

Results:

At lower substrate concentration, metabolism of voriconazole was higher with higher relative formation of N-oxide metabolite (Figure S1 A and B). In presence of CYP2C9, 2C19 and 3A4/5 selective inhibitor sulfaphenazole, ticlopidine and ketoconazole, respectively metabolism of voriconazole was inhibited and indicating the involvement of CYP2C9, 2C19 and 3A4/5 enzymes in metabolism. We observed that the presence of 2C19 and 3A4/5 selective inhibitor ticlopidine and ketoconazole, respectively have inhibitory effect on voriconazole N-oxide formation, although no effect observed in presence of CYP2C9 selective inhibitor sulfaphenazole. (Figure S1B).

We observed formation of 4-OH-diclofenac, 4-OH-S-mephenytoin and 1-OH-Midazolam by incubation of CYP2C9 probe substrate diclofenac, CYP2C19 probe substrate S-mephenytoin and CYP3A4/5 substrate midazolam, respectively in mouse liver microsomes. It is well known that metabolite 4-OH-diclofenac, 4-OH-S-mephenytoin and 1-OH-Midazolam are generated via marker biotransformation reactions mediated through CYP 2C9, 2C19 and 3A4/5 in human liver microsomes. Hence presence of these metabolites in mouse liver microsomal incubated samples indicated the presence of human CYP2C9, 2C19 and 3A4/5 orthologs in mouse.

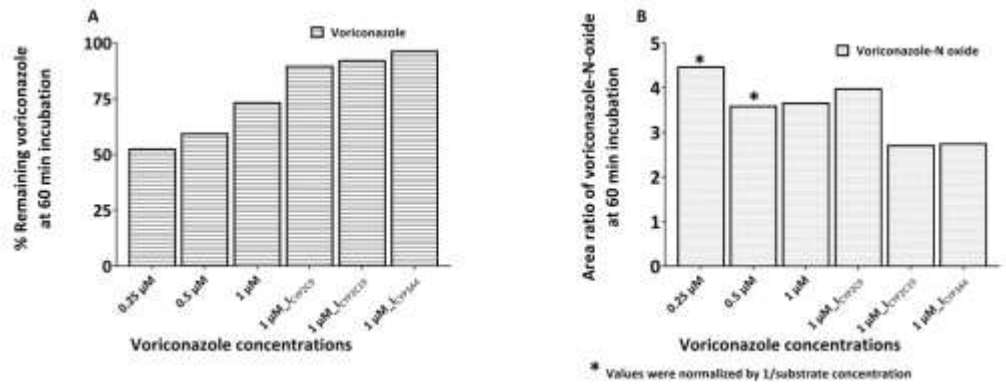

Figure S1: In vitro metabolic stability of voriconazole in mouse liver microsomes; (A) Concentration dependent metabolism of voriconazole and effect of CYP2C9, 2C19 and 3A4/5 selective inhibitor sulfaphenazole, ticlopidine and ketoconazole on metabolism; (B) Concentration dependent formation of metabolite voriconazole N-oxide and effect of CYP2C9, 2C19 and 3A4/5 selective inhibitor sulfaphenazole, ticlopidine and ketoconazole on voriconazole N-oxide formation.

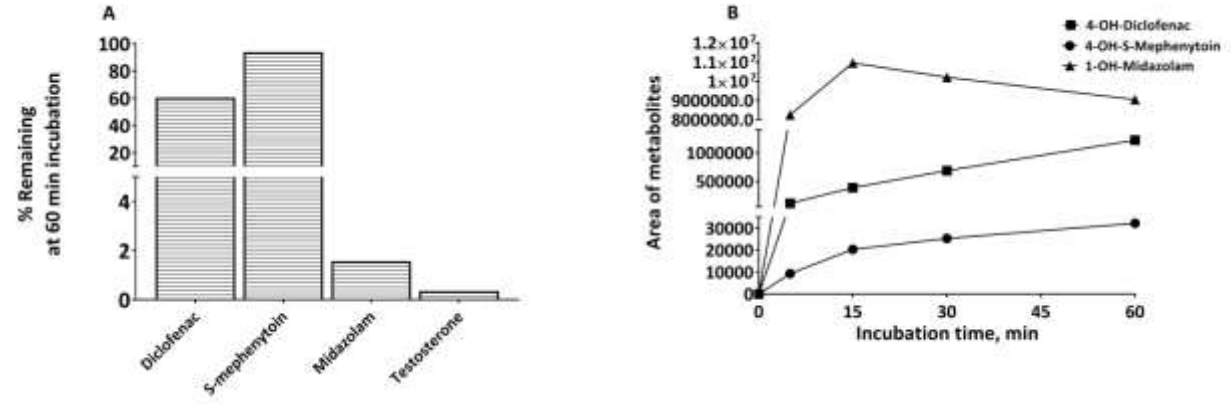

Figure S2: investigation of presence of orthologs of human CYP2C9, 2C19 and 3A4/5 in mouse; (A) In vitro metabolic stability of positive control substrate testosterone, CYP2C9 probe substrate diclofenac, CYP2C19 probe substrate S-mephenytoin and CYP3A4/5 substrate midazolam; (B) Formation of metabolites, 4-OH-diclofenac, 4-OH-S-mephenytoin and 1-OH-Midazolam by incubation of diclofenac, S-mephenytoin and midazolam, respectively in mouse liver microsomes with incubation time.