Supplementary Methods

Part A

Estimation of Michaelis-Menten constant (J_{max} and K_m) of milademetan for P-gp transport in human Caco-2 cell monolayer systems

The Michaelis-Menten constant (J_{max} and K_m) of milademetan for P-gp transport was determined using P-gp transport assay performed in human Caco-2 cell monolayers. In the bidirectional transport assay of [^{14}C]milademetan, the culture medium on both the donor and receiver sides was replaced with Hanks' Balanced Salt Solution (HBSS) buffer and preincubated for 15 minutes at 37°C. The HBSS buffer on the receiver side was then replaced with fresh HBSS buffer, while the HBSS buffer on the donor side was replaced with HBSS buffer containing 1, 3, 10, 30, and 100 µM of [^{14}C]milademetan. A typical potent P-gp/BCRP inhibitor, GF120918 (Elacridar) 5 µM, was also added to both donor and receiver sides to confirm the effect on P-gp in the bidirectional transport assay. After incubation for 120 minutes at 37°C, aliquots of the solution from the receiver side were collected, and radioactivity was measured with a liquid scintillation counter (LSC), 2300TR (PerkinElmer, Inc).

The permeability coefficient (P_{app}) and P_{app} ratio of milademetan were calculated according to the publication by Mikkaichi *et al*.[1] and were summarized in the Table below.

$$P_{app} = \frac{1}{C0A} \cdot \frac{dQ}{dt}$$

$$P_{app}Ratio = \frac{P_{app,B\ to\ A}}{P_{app,A\ to\ B}}$$

where dQ/dt is the steady-state appearance rate of the substrate on the receiver side (dpm/s), C0 is concentration of the test compound on the donor side (dpm/mL), and A is the surface area of the monolayer (0.33 cm^2); and $P_{app,A\ to\ B}$ is the P_{app} value from the apical-to-basal direction and $P_{app,B\ to\ A}$ is the P_{app} value from the basal-to-apical direction.

P_{app} values and P_{app} ratios of milademetan in Caco-2 cell monolayers

Milademetan (µM)	Inhibitor	$P_{app,AtoB}$ (10^{-6}cm/s)	$P_{app,BtoA}$ (10^{-6}cm/s)	P_{app} ratio (B to A/A to B)
1	–	1.13	18.2	16.11
3	–	1.24	17.5	14.11
10	–	1.86	17.3	9.30
30	–	3.66	9.95	2.72
100	–	4.42	5.79	1.31
1	GF120918 (5 µM)	5.07	3.44	0.68

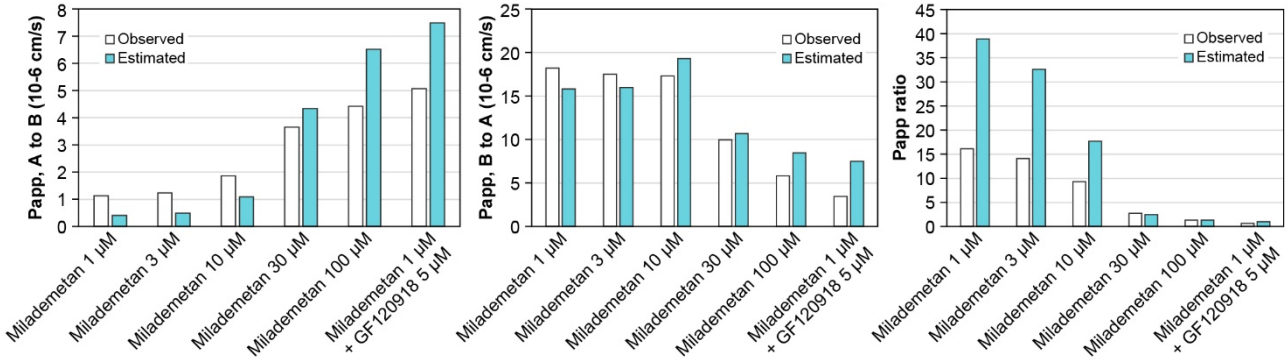
To obtain a single set of parameter estimates for K_m value, maximum velocity (J_{max}), and passive diffusion (CL_{PD}), the kinetic analysis using a 3-compartment model reported by Tachibana *et al*[2] was performed. The 3-compartment model consisted of apical, cellular, and basal compartments under the assumption that the passive permeability across apical and basal membranes is identical, and the model was fitted simultaneously to the P_{app} values both in apical-to-basal and basal-to-apical directions. In the

case where the data were obtained in the presence of the P-gp inhibitor (GF120918 5 µM), the J_{max} value was set as zero. As shown in the table below, K_m, J_{max}, and passive diffusion were calculated to be 0.324 µM, 13.4 pmol/min/cm^2, and 17.0 × 10^{-6}cm/s, respectively. The P_{app} and P_{app} ratios are reasonably represented by the 3-compartment model shown in the figure below.

Milademetan P-gp transport kinetic parameter estimates in Caco-2 cell monolayers

Parameter	Unit	Value	CV (%)
Km	µM	0.324	1.3
Vmax	pmol/min/cm^2	13.4	7.8
P_{AC}=P_{CA}=P_{BC}=P_{CB}	10^{-6} cm/s	17.0	3.9

Model-fitting results of $P_{app,A\ to\ B}$ (A), $P_{app,\ B\ to\ A}$ (B), and $P_{app\ ratio}$ (C) of milademetan in Caco-2 cells



References

1. Mikkaichi T, Yoshigae Y, Masumoto Y, et al. Edoxaban transport via p-glycoprotein is a key factor for the drug's disposition. *Drug Metab Dispos*. 2014;42:520-528.
2. Tachibana T, Kitamura S, Kato M, et al. Model analysis of the concentration-dependent permeability of p-gp substrates. *Pharm Res.* 2010;27:442-446.

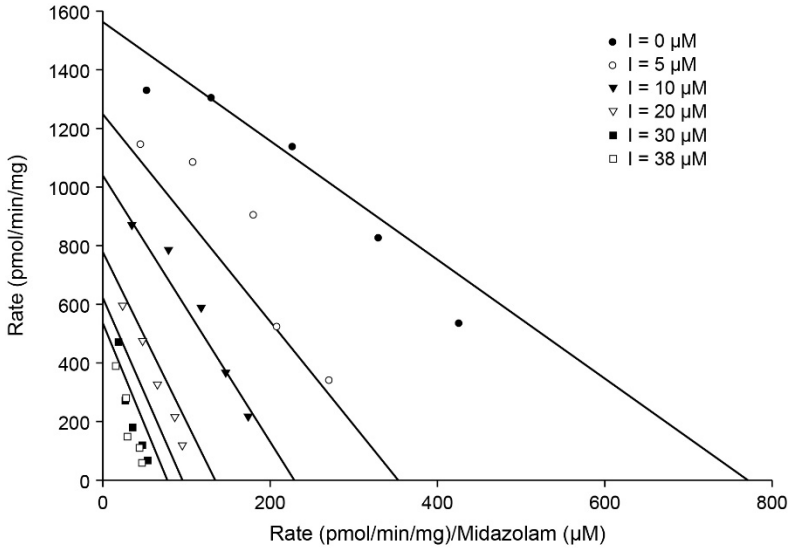
Supplementary Methods

Part B

Estimation of inhibitory parameters (K_i, K_{app}, and K_{inact}) of milademetan on CYP450 isoenzymes using human hepatic microsomes

The inhibitory potential of milademetan on the activities of human hepatic CYP isoenzymes were characterized *in vitro* using pooled human hepatic microsomes. To determine the direct inhibitory effect of milademetan on CYP3A, the *in vitro* assays were conducted at 5 concentrations of midazolam (1.25, 2.5, 5, 10, and 25 µM) and 6 concentrations of milademetan (0, 5, 10, 20, 30, and 38 µM). Four modified Michaelis-Menten equations (competitive inhibition, mixed inhibition, noncompetitive inhibition, and uncompetitive inhibition) were fitted to CYP3A activity. The inhibition type was determined to be the combination of competitive and uncompetitive inhibition, and the potency of inhibition (K_i) was calculated to be 4.2 µM, shown in the Eadie-Hofstee plot below.

Eadie-Hofstee plot of midazolam metabolism at milademetan concentrations of 0, 5, 10, 20, 30, and 38 µM

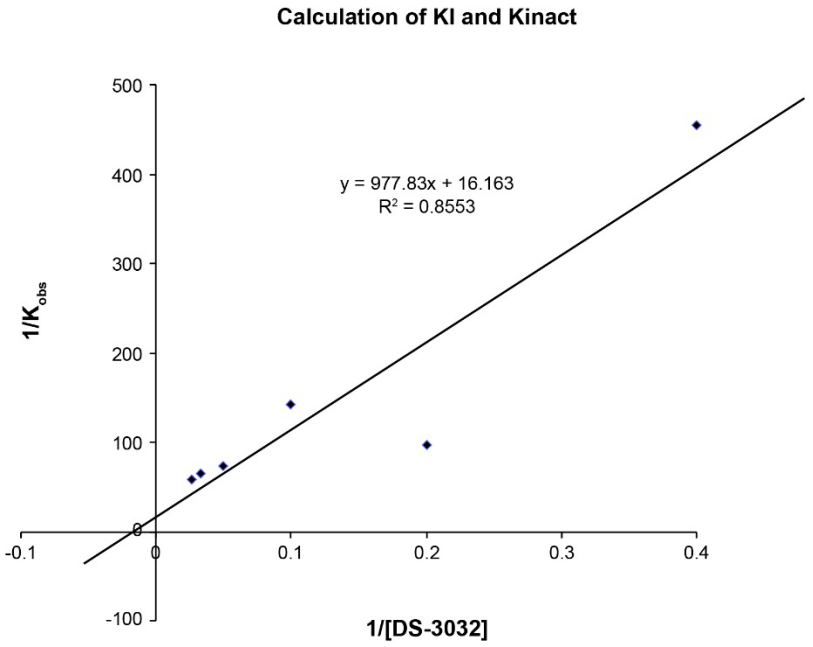


V_{max} = 1560 pmol/min/mg
K_m = 2 µM
K_i = 4.2 µM
α = 4.7

To determine the kinetics of metabolism-dependent inhibition, milademetan 0, 2.5, 5, 10, 20, 30, and 38 µM was preincubated in the absence and presence of nicotinamide adenine dinucleotide phosphate (NADPH) (1 mM) for 0, 5, 10, 15, 20, and 30 minutes. The data were processed for calculation of K_{app} and K_{inact} based on the method of Maurer and Fung (2000).[1] The preincubation time for each concentration of milademetan was plotted against the remaining CYP3A activity as percent of control normalized with those obtained in the absence of NADPH in logarithmic scale. The equation was then obtained for each linear curve. The slope of each curve (the exponent of e) representing the observed inactivation

constant (K_{obs}) was recorded. K_{app} 60.5 µM and K_{inact} 3.71 h^{-1} were derived based on the linear correlation of the reciprocal of K_{obs} and the reciprocal of milademetan concentration, shown in the figures below.

Relationship between observed rates of inactivation and milademetan concentrations for CYP3A4/5 activity (midazolam 1'-hydroxylase)



K_I = 60.5 µM and k_{inact} = 0.0619 $minute^{-1}$

Reference

1. Maurer TS, Fung HL. Evaluation of nitric oxide synthase activity and inhibition kinetics by chemiluminescence. *Nitric Oxide*. 2000;4:372-378.

Supplementary Tables

Table S1 Model parameter input for the internally modified itraconazole model (SV-Itraconazole_Fasted Soln, V17)

Parameter (units)	Definition	Values
Molecular weight (g/mol)		705.6
logP	Octanol to water partition coefficient	4.47
Compound type		Monoprotic base
pKa	Ionization coefficient	4.28
B/P	Blood to plasma ratio	0.58
f_u	Unbound fraction	0.016
Main plasma-binding protein		Human serum albumin
$f_{u,gut}$	Unbound fraction in the gut	0.016
Distribution model		Minimal PBPK model
V_{ss} (L/kg)	Volume of distribution at steady state	2.520463
Enzyme		CYP1A2
Pathway		OH
CL_{int} (µL/min/pmol)	Intrinsic clearance	1
Enzyme		CYP3A4
Pathway		OH
V_{max} (pmol/min/pmol)	Maximal metabolism rate	0.065
K_m (µM)	Michaelis-Menten constant	0.0039
CL_R (L/h)	Renal clearance	0
Enzyme		CYP3A4
K_i (µM)	CYP3A4 inhibition constant	0.0013
Transporter		ABCB1 (P-gp/MDR1)
Organ		Gut
K_i (µM)	P-gp inhibition constant	0.03
Transporter		ABCB1 (P-gp/MDR1)
Organ		Liver

K_i (μM)	P-gp inhibition constant	0.03

CYP3A4, cytochrome P450 3A4; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein.

Table S2 Model parameter input for the itraconazole metabolite model (SV-OH-Itraconazole, V17)

Parameter (units)	Definition	Values
Molecular weight (g/mol)		721.7
logP	Octanol to water partition coefficient	4.47
Compound type		Monoprotic base
pKa	Ionization coefficient	4.28
B/P	Blood to plasma ratio	0.58
f_u	Unbound fraction	0.016
Main plasma-binding protein		Human serum albumin
$f_{u,gut}$	Unbound fraction in the gut	0.016
Distribution model		Minimal PBPK model
V_{ss} (L/kg)	Volume of distribution at steady state	1.03532
Enzyme		CYP3A4
Pathway		Pathway 1
Vmax (pmol/min/pmol)	Maximal metabolism rate	0.13
Km (μM)	Michaelis-Menten constant	0.027
CL_R (L/h)	Renal clearance	1.39
Enzyme		CYP3A4
Ki (μM)	CYP3A4 inhibition constant	0.0023

CYP3A4, cytochrome P450 3A4; PBPK, physiologically based pharmacokinetic.

Table S3 Physicochemical and pharmacokinetic parameter input for PBPK model of posaconazole

Parameter (units)	Definition	Values
Molecular weight (g/mol)		700.792
logP	Octanol to water partition coefficient	4
Compound type		Diprotic base
pKa	Ionization coefficient	2.88, 4.11
B/P	Blood to plasma ratio	0.62
f_u	Unbound fraction	0.01
Main plasma-binding protein		Human serum albumin
f_a	Fraction absorbed	0.85
ka (1/h)	Absorption rate constant	0.55
Lag time (h)		0.8
$f_{u,gut}$	Unbound fraction in the gut	1
Q_{gut} (L/h)	Nominal flow through the gut	16.992
Distribution model		Minimal PBPK model
V_{ss} (L/kg)	Volume of distribution at steady state	2.96
CL_{iv} (L/h)	Systemic clearance	7.32
CL_R (L/h)	Renal clearance	0
Enzyme		CYP3A4
K_i (μM)	CYP3A4 reversible inhibition constant	0.005

CYP3A4, cytochrome P450 3A4; PBPK, physiologically based pharmacokinetic.

Table S4 Trial design for simulation of DDI effects of moderate CYP3A inhibitors

Sample size and population	Milademetan	Interacting drugs
Sim-Healthy Volunteers: 10 trials, 10 subjects in each trial Age: 18–55 years Female: 0.5	100 mg SD on day 7, simultaneous administration with fluconazole Fasted conditions	Fluconazole 400 mg on day 1, 200 mg QD on days 2–13
	100 mg SD on day 7, 2 hours after the dose of erythromycin Fasted conditions	Erythromycin 500 mg TID on days 1–13
	100 mg SD on day 7, simultaneous administration with verapamil Fasted conditions	Verapamil 100 mg TID on days 1–13

CYP3A, cytochrome P450 3A; DDI, drug-drug interaction; QD, once daily; SD, single dose; TID, 3 times daily.

Table S5 Trial design for simulation of washout period after discontinuation of strong CYP3A inhibitors itraconazole and posaconazole

Sample size and population	Scenario	Milademetan	Interacting drugs
Sim-Healthy Volunteers Age: 20 years Male	1	80 mg QD on days 1–8 160 mg QD on days 9–14 Fasted conditions	Itraconazole 200 mg BID on day 1, 200 mg QD on days 2–8 1 hour before milademetan dose
	2	80 mg QD on days 1–10 160 mg QD on days 11–14 Fasted conditions	
	3	80 mg QD on days 1–14 Fasted conditions	
	4	80 mg QD on days 1–8 160 mg QD on days 9–14 Fasted conditions	Posaconazole 200 mg TID on days 1–8 2 hours before milademetan dose
	5	80 mg QD on days 1–10 160 mg QD on days 11–14 Fasted conditions	
	6	80 mg QD on days 1–14 Fasted conditions	
	7	160 mg QD on days 1–14 Fasted conditions	None

BID, twice daily; CYP3A4, cytochrome P450 3A4; TID, 3 times daily; QD, once daily.

Table S6 Summary of predicted PK parameters for the interaction of milademetan with fluconazole, erythromycin, and verapamil

	AUC_{inf} (ng/mL•h)	C_{max} (ng/mL)	AUCR	$C_{max}R$
Inhibitory effect of fluconazole on PK of milademetan				
Control	14,015	711		
With fluconazole	24,143	804	1.72 (1.69–1.76)	1.13 (1.12–1.14)
Inhibitory effect of erythromycin on PK of milademetan				
Control	14,411	723		
With erythromycin	27,537	805	1.91 (1.83–1.99)	1.11 (1.10–1.12)
Inhibitory effect of verapamil on PK of milademetan				
Control	14,384	766		
With verapamil	29,011	893	2.02 (1.93–2.11)	1.17 (1.15–1.18)

AUC_{inf} and C_{max} are presented as geometric mean. AUCR and $C_{max}R$ are presented as geometric mean (90% CI).

AUC_{inf}, concentration-time curve from zero to infinity; AUCR, area under the concentration-time curve ratio; C_{max}, maximum serum concentration; $C_{max}R$, maximum serum concentration ratio; PK, pharmacokinetics.

Table S7 Simulated milademetan AUC_{tau} before and after discontinuation of strong CYP3A inhibitors itraconazole and posaconazole

Scenario	Milademetan AUC_{tau} (ng/mL•h)						
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	23,172 (1.12)	32,126[a] (1.56)	34,959 (1.69)	28,614 (1.39)	24,452 (1.18)	22,652 (1.10)	21,735 (1.05)
2	23,172 (1.12)	23,364 (1.13)	22,093 (1.07)	22,929[a] (1.11)	21,638 (1.05)	21,163 (1.03)	20,923 (1.01)
3	23,172 (1.12)	23,364 (1.13)	22,093 (1.07)	16,378 (0.79)	13,084 (0.63)	11,614 (0.56)	10,879 (0.53)
4	20,323 (0.98)	28,513[a] (1.38)	30,919 (1.50)	30,368 (1.47)	28,276 (1.37)	25,959 (1.26)	24,077 (1.17)
5	20,323 (0.98)	20,207 (0.98)	19,044 (0.92)	24,624[a] (1.19)	25,096 (1.22)	24,182 (1.17)	23,097 (1.12)
6	20,323 (0.98)	20,207 (0.98)	19,044 (0.92)	17,146 (0.83)	15,073 (0.73)	13,311 (0.64)	12,056 (0.58)
7	20,495	20,553	20,591	20,613	20,627	20,636	20,641

Simulation scenario is defined in **Table S5**. Values in parentheses are fold of change of AUC_{tau} to AUC_{tau} on day 14 of scenario 7.

AUC_{tau}, area under the plasma concentration-time curve over dosing interval; CYP3A, cytochrome P450 3A.

[a] Day when milademetan dose was returned to 160 mg QD.