SUPPLEMENTARY MATERIAL TO:

In Vitro Assessment of Time-Dependent Inhibitory Effects on CYP2C8 and CYP3A Activity by Fourteen Protein Kinase Inhibitors

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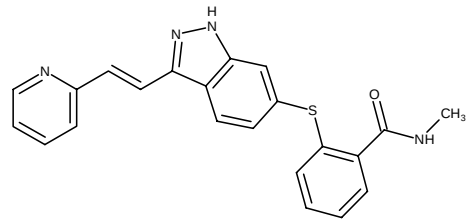
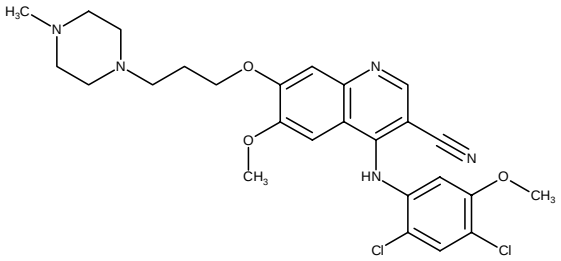
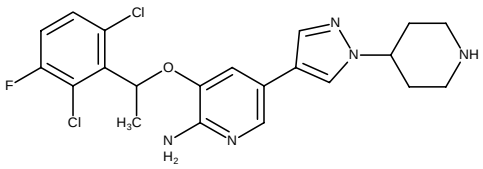
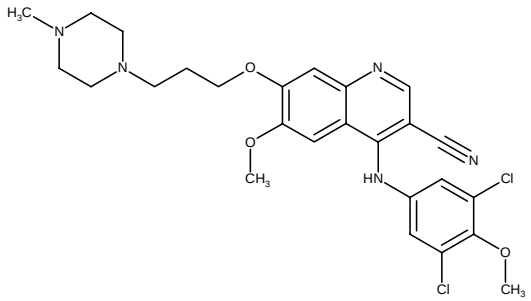
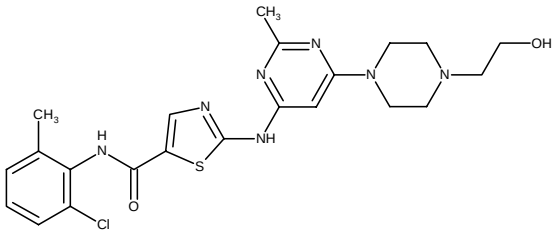
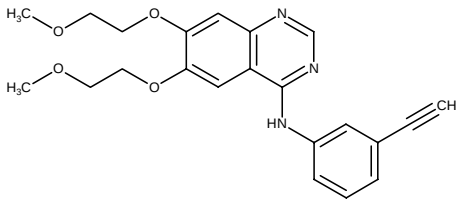
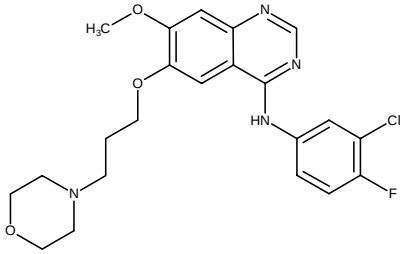
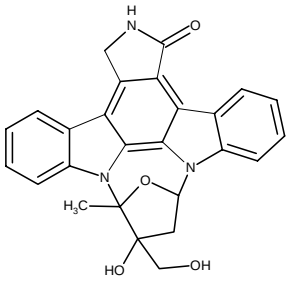
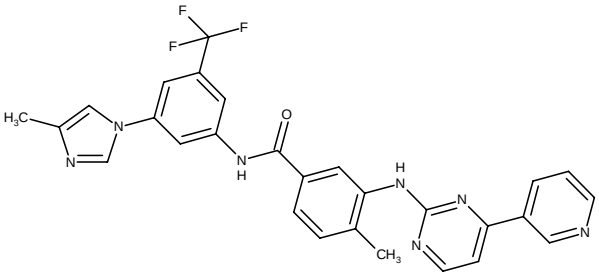
 Axitinib (Inlyta)	 Bosutinib (Bosulif)
 Crizotinib (Xalkori)	 Bosutinib isomer 1
 Dasatinib (Sprycel)	 Erlotinib (Tarceva)
 Gefitinib (Iressa)	 Lestaurtinib



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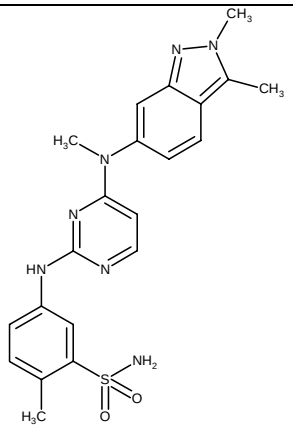

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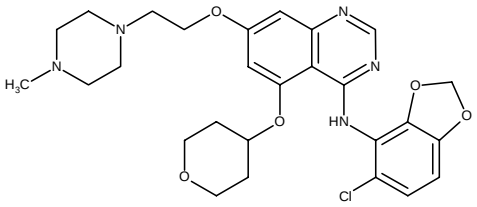

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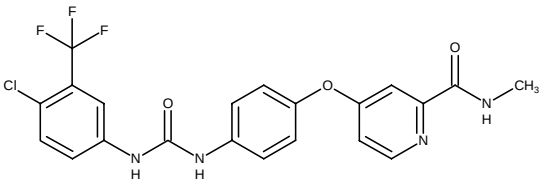

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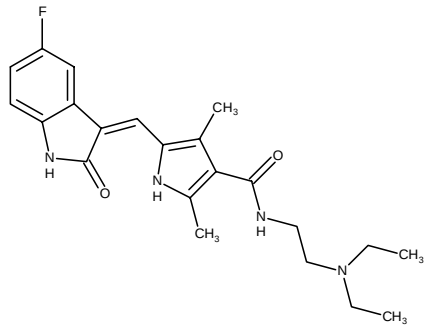

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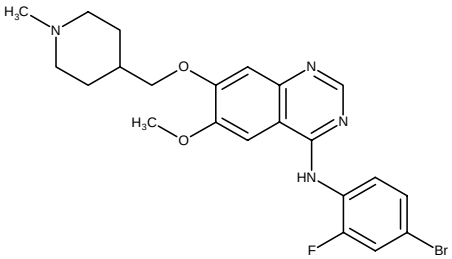
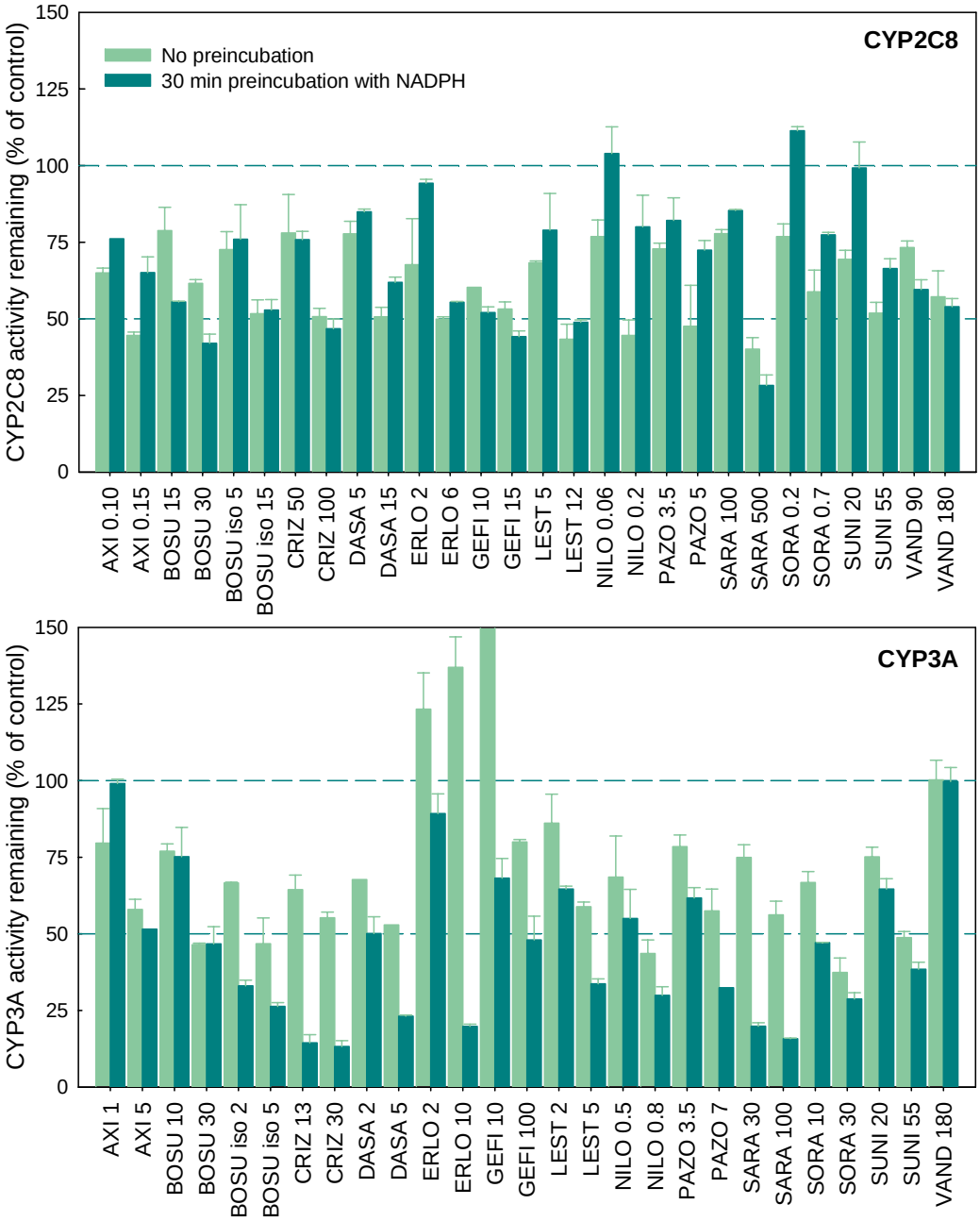


Vandetanib (Caprelsa)

Supplementary Figure S1. Molecular structures of the fourteen compounds tested in the present study.



Supplementary Figure S2. Effects of fourteen protein kinase inhibitors on amodiaquine N-deethylation (CYP2C8 marker reaction; top figure) and midazolam 1′-hydroxylation (CYP3A marker reaction; bottom figure) with or without a 30 min preincubation in the presence of NADPH. The micromolar concentrations of the inhibitors are given. Incubations were conducted in HLM (0.1 mg/ml protein) with 2 µM substrate. Data points are mean + SD values of duplicate incubations. Abbreviations: AXI, axitinib; BOSU, bosutinib; BOSU iso, bosutinib isomer 1; CRIZ, crizotinib; DASA, dasatinib; ERLO, erlotinib; GEFI, gefitinib; LEST, lestaurtinib; NILO, nilotinib; PAZO, pazopanib; SARA, saracatinib; SORA, sorafenib; SUNI, sunitinib; VAND, vandetanib.

Supplementary Table S1. Parameters used in the static predictions of drug-drug interaction potential based on in vitro inhibition data.

Kinase inhibitor	Standard dosing regimen	Steady state C_{max} (ng/ml)	Steady state C_{trough} (ng/ml)	Molecular weight (g/mol)	f_u	k_a (1/h)	References
Axitinib	5 mg BID	~30	n/a	386.47	0.004	0.40[a]	http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202324lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202324Orig1s000ClinPharmR.pdf, Rugo *et al.* (2005)
Bosutinib	500 mg OD	273	n/a	530.45	0.04	0.61	http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203341lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf, Daud *et al.* (2012)
Gefitinib	250 mg OD	386 (after 225 mg OD)	n/a	446.9	0.09	0.52	http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/021399s008lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-399_IRESSA_Clinr.pdf, Schaiquevic *et al.* (2008)
Lestaurtinib	80 mg BID (used in trials)	12117	n/a	439.46	<0.01	1.0[a]	http://aml17.cardiff.ac.uk/files/aml17_protocolv2.pdf
Saracatinib	175 mg OD (used in trials)	444	215	542.03	0.10	0.25[a]	Baselga *et al.* (2010); Tou and Chen (2012)

Abbreviations: BID, twice daily; C_{max}, peak concentration; C_{trough}, trough concentration; f_u, unbound fraction; k_a, absorption rate; n/a, not available; OD, once daily.
[a] Estimated as $1/T_{max}$ from clinical data.

Supplementary Table S2. Prediction of the maximal effect of direct and mechanism-based inhibition on the pharmacokinetics of CYP2C8 and CYP3A substrates by five protein kinase inhibitors.

Kinase inhibitor	Dose	$C_{max,u,hep,inlet}$ (µM)	C_{max} [a] (µM)	$C_{max,u}$ (µM)	I_g (µM)	Victim drug [b]	Predicted $AUC_{po,i}$ / AUC_{po} [c]	
							based on $C_{max,u,hep,inlet}$	based on $C_{max,u}$ (µM)
Axitinib	5 mg BID	0.00052	0.078	0.00032	0.28	CYP2C8 substrate	<1.1	<1.1
						CYP3A substrate	<1.1 (<1.1)	<1.1 (<1.1)
Bosutinib	500 mg OD	0.23	0.51	0.021	31	CYP2C8 substrate	1.2	<1.1
						CYP3A substrate	1.1 (1.9)	<1.1 (1.8)
Gefitinib	250 mg OD	0.32	0.86	0.078	16	CYP2C8 substrate	1.1	<1.1
Lestaurtinib	80 mg BID (used in trials)	0.29	28	0.28	9.8	CYP2C8 substrate	1.1	1.1
						CYP3A substrate	2.8 (4.9)	2.7 (4.7)
Saracatinib	175 mg OD (used in trials)	0.16	0.82	0.082	4.3	CYP2C8 substrate	<1.1	<1.1
						CYP3A substrate	5.5 (8.4)	3.2 (5.5)

Abbreviations: AUC, area under the plasma concentration time curve; BID, twice daily; $C_{max,u,hep,inlet}$, unbound peak hepatic inlet concentration; C_{max}, peak concentration; $C_{max,u}$, unbound peak concentration; I_g, intestinal concentration; n/a, not available; OD, once daily.

[a] Steady-state C_{max} from Supplementary Table S1 as converted to µM.

[b] Substrates were assumed to be completely eliminated by the enzyme in question (i.e. fm_{CYP2C8} = 1 for CYP2C8 substrates and fm_{CYP3A} = 1 for CYP3A substrates).

[c] Predicted $AUC_{po,i}$ / AUC_{po} when direct and potential mechanism-based inhibition of hepatic CYP2C8 or CYP3A are considered and unbound hepatic peak inlet or peak concentrations of the inhibitor are used. Within brackets: predicted change in the AUC of midazolam (fm_{CYP3A} = 0.94, F_g = 0.51), when inhibition of intestinal CYP3A is included in the prediction.

Supplementary Table S3. Fourteen protein kinase inhibitors and their inhibitory effects on CYP2C8 activity in HLM incubations as reported in the literature and in the present study.

Kinase inhibitor	Direct IC_{50} (substrate)	TDI IC_{50} (substrate)	K_i (substrate)	K_I (substrate)	k_{inact} (substrate)	TDI	Comment	References
Axitinib (Inlyta)	-	-	0.5 µM (P)	-	-	n/i		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202324Orig1s000ClinPharmR.pdf
	-	-	0.17 µM (P)	-	-	no		Wang *et al.* (2014)
	0.11 µM (A)	0.24 µM (A)	-	-	-	no		Present study
Bosutinib (Bosulif)	-	-	-	-	-	n/i	Little direct inhibition at 200 µM[a]	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000PharmR.pdf
	-	-	1.94 µM (P)	-	-	no		Wang *et al.* (2014)
	43.3 µM (A)	16.9 µM (A)	-	54.8 µM (A)	0.018 (A)	yes		Present study
Bosutinib isomer 1	-	-	-	-	-	no	~50% direct inhibition at 15 µM	Present study
Crizotinib (Xalkori)	>30 µM (A)	-	-	-	-	n/i	Does not inhibit CYP2C8	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf
	-	-	-	-	-	no	50% direct inhibition at 100 µM	Present study
Dasatinib (Sprycel)	12 µM (P)	-	3.6 µM (P)	-	-	n/a		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021986s000_SprycelClinPharmR.pdf
	38.6 µM (P)	-	-	-	-	n/i		Kim *et al.* (2013)
	-	-	6.31 µM (P)	-	-	no		Wang *et al.* (2014)
	-	-	-	-	-	no	~50% direct inhibition at 15 µM	Present study
Erlotinib (Tarceva)	-	-	-	-	-	n/i		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-743_Tarceva.cfm
	6.17 µM (P)	-	5.8 µM (P)	-	-	n/i		Dong *et al.* (2011)
	9.5 µM (P)	-	-	-	-	n/i		Kim *et al.* (2013)
	-	-	4.02 µM (P)	-	-	possibly	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no	Wang *et al.* (2014)

							preincubation	
	-	-	-	-	-	no	~50% direct inhibition at 6 µM	Present study
Gefitinib (Iressa)	31.0 µM (P)	-	-	-	-	n/i		Kim *et al.* (2013)
	-	-	8.69 µM	-	-	possibly	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	12.3 µM (A)	10.2 µM (A)	-	-	-	no		Present study
Lestaur-tinib (CEP-701)	9.5 µM (A)	7.6 µM (A)	-	-	-	no		Present study
Nilotinib (Tasigna)	<1 µM (P)	-	0.236 µM (n/a)	-	-	no		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068s000_ClinPharmR.pdf
	0.7 µM (A), 0.4 µM (P), 7.5 µM (R)	-	0.9 µM (P), 0.15 µM (A)	-	-	n/i		Kim *et al.* (2013)
	-	-	0.10 µM (P)	-	-	no		Wang *et al.* (2014)
	-	-	-			no	~50% direct inhibition at 0.2 µM	Present study
Pazopanib (Votrient)	10 µM (P)	13 µM (P)	-	-	-	no		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_ClinPharmR.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_PharmR.pdf
	-	-	3.72 µM (P)	-	-	no		Wang *et al.* (2014)
	-	-	-			no	50% direct inhibition at 5 µM	Present study
Saracatinib (AZD0530)	202 µM (A)	172 µM (A)	-	-	-	no	No TDI	Present study

Sorafenib (Nevaxar)	-	-	1-2 µM[a] (n/a)	-	-	n/a		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021923_s000_Nexavar_BioPharmR.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021923_s000_Nexavar_PharmR.pdf
	-	-	2.4 µM[a] (n/a)	-	-	n/a		Flaherty *et al.* (2011)
			1.59 µM (P)	-	-	possibly	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	no	~40% direct inhibition at 0.7 µM	Present study
Sunitinib (Sutent)	-	-	28 µM (P)	-	-	n/a		Product information
	-	-	91.51 µM (P)	-	-	no		Wang *et al*. (2014
	-	-	-	-	-	no	~50% direct inhibition at 55 µM	Present study
Vandetanib (Caprelsa)	-	-	-	-	-	no	57% inhibition at 100 µg/ml (210 µM)	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022405Orig1s000ClinPharmR.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022405Orig1s000PharmR.pdf
	-	-	-	-	-	no	~50% inhibition at 180 µM	Present study

Abbreviations: A, amodiaquine; n/a, not available; n/i, not investigated; P, paclitaxel; R, rosiglitazone; TDI, time-dependent inhibition; TDI IC_{50}, IC_{50} following preincubation with NADPH of the inhibitor.

[a] Unclear whether the study was performed using HLM or recombinant enzymes.

Supplementary Table S4. Fourteen protein kinase inhibitors and their inhibitory effects on CYP3A activity in HLM incubations as reported in the literature and in the present study.

Kinase inhibitor	Direct IC_{50} (substrate)	TDI IC_{50} (substrate)	K_i (substrate)	K_I (substrate)	k_{inact} (substrate)	TDI	Comment	References
Axitinib (Inlyta)	-	-	8.3 µM (T)	-	-	n/a		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202324Orig1s000ClinPharmR.pdf
	-	-	1.94 µM (P)	0.93 µM (P)	0.0137 1/min	yes		Wang *et al.* (2014)
	6.6 µM (M)	4.8 µM (M)	-	-	-	no	TDI excluded as IC_{50} ratio <1.5	Present study
Bosutinib (Bosulif)	-	-	27 µM (n/a)[a]	-	-	no		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf
	-	-	0.14 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	22 µM (M)	20 µM (M)	-		-	no		Present study
Bosutinib isomer 1	-	-	-	-	-	likely	~50% direct and ~75% NADPH- and time-dependent inhibition at 5 µM	Present study
Crizotinib (Xalkori)	8.2 µM (F), >30 µM (M), 7.3 µM (T)	-	-	3.0 µM (M)	0.11 1/min (M)	yes		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf
	-	-	-	0.37 µM (M)	0.115 1/min (M)	yes		Mao *et al.* (2013)
	-	-	-	-	-	likely	~50% direct and >85% NADPH- and time-dependent inhibition at 30 µM	Present study
Dasatinib (Sprycel)	18 µM (M), 10 µM (T)	-	-	1.9 µM (n/a)	0.022 1/min (n/a)	yes		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021986s000_Sprycel_ClinPharmR.pdf
	-	-	-	6.3 µM (M)	0.034 1/min (M)	yes		Li *et al.* (2009)

	-	0.6 µM (T)	-	2.6 µM (M)	0.024 1/min (M)	yes		Kenny *et al.* (2012)
	23.7 µM (M)	-	-	-	-	n/i		Kim *et al.* (2013)
	-	-	2.29 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	likely	~50% direct and >75% NADPH- and time-dependent inhibition at 5 µM	Present study
Erlotinib (Tarceva)	-	-	8.0 µM (n/a)	-	0.009 1/min (M)	n/a	Complex inhibition	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-743_Tarceva_biopharmr.PDF
	-	10.6 µM (M)	-	7.5 µM (M)	0.009 1/min (M)	yes	Stimulation of midazolam 1'-hydroxylation in direct inhibition experiments	Calvert *et al.* (2014)
	31.3 µM (T), 20.5 µM (N)	-	14.1 µM (T), 4.3 µM (N)	6.3 µM (M), 9.0 µM (T), 10.1 µM (N)	0.035 1/min (M), 0.045 1/min (T), 0.058 1/min (N)	yes	Substrate-dependent modulation with both stimulation and inhibition: stimulation of midazolam 1'-hydroxylation when NADPH was excluded from preincubation	Dong *et al.* (2011)
	-	1.5 µM (T)	-	8.2 µM (M)	0.057 1/min (M)	yes		Kenny *et al.* (2012)
	54.8 µM (M)	-	-	-	-	n/i		Kim *et al.* (2013)
	-	-	-	22 µM (M)	0.09 1/min (M)	yes		Li *et al.* (2010)
	-	-	-	-	-	n/i	Stimulation of midazolam 1'-hydroxylation	Li *et al.* (2007)
	-	-	1.28 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	likely	Stimulated	Present study

							midazolam 1'-hydroxylation when NADPH was excluded from preincubation, >75% NADPH- and time-dependent inhibition at 10 µM	
Gefitinib (Iressa)	-	-	-	-	-	n/a	<10% inhibition of testosterone 6β-hydroxylation at 11.2 µM gefitinib	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-399_IRESSA_Clinr.pdf
	-	-	-	-	-	n/i	Stimulation of midazolam and triazolam 1'-hydroxylation at 1-20 µM gefitinib	Fujita *et al.* (2005) Li *et al.* (2007) van Waterschoot *et al.* (2009)
	-	4.8 µM (T)	-	14.1 µM (T)	0.019 1/min (T)	yes		Kenny *et al.* (2012)
	-	-	4.80 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	likely	10 µM stimulated midazolam 1'-hydroxylation when NADPH was excluded from preincubation, ~50% NADPH- and time-dependent inhibition at 100 µM	Present study
Lestaurtinib (CEP-701)	-	-	<5 µM (n/a)	-	-	n/i	TDI not investigated	http://aml17.cardiff.ac.uk/files/aml17_protocolv2.pdf
	-	-	5.2 µM (M), 4.3 µM (T)	-	-	n/i	In contrast to HLM data, lestaurtinib increased midazolam 1′-hydroxylation in hepatocytes concentration-dependently, but inhibited 6β-hydroxylation of	http://issx.confex.com/issx/15na/webprogram/Paper11788.html

							testosterone in a concentration-independent manner in hepatocytes	
	4.7 µM (M)	2.1 µM (M)	-	30.7 µM (M)	0.040 1/min (M)	yes		Present study
Nilotinib (Tasigna)	1 µM (M), 1 µM (T)	-	0.448 µM (n/a)	-	-	no		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068s000_ClinPharmR.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068s000_PharmR_P1.pdf
	-	0.4 µM (T)	-	1.5 µM (M)	0.033 1/min (M)	yes	TDI	Kenny *et al.* (2012)
	-	-	0.28 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	likely	~50% direct inhibition and ~70% NADPH- and time-dependent inhibition at 0.8 µM	Present study
Pazopanib (Votrient)	11 µM (At), 12 µM (M), 14 µM (N)	6.8 µM (At), 8.9 µM (M), 8.1 µM (N)	-	-	-	no		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_ClinPharmR.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_PharmR.pdf
	-	0.3 µM (T)	-	2.9 µM (M)	0.021 1/min (M)	yes		Kenny *et al.* (2012)
	-	-	0.97 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	likely	~50% direct and 70% NADPH- and time-dependent inhibition at 7 µM	Present study
Saracatinib	-	-	-	-	-	n/a	AZD0530 is a	http://www.ncats.nih.go

(AZD0530)							moderately potent CYP3A4 inhibitor	v/files/AZD0530.pdf
	46 µM (M)	1.8 µM (M)	-	12.6 µM (M)	0.096 1/min (M)	yes		Present study
Sorafenib (Nevaxar)	-	-	29 µM (n/a)	-	-	n/a		http://www.nexavar.com/html/download/Nexavar_PI.pdf
	-	-	4.9[a] (n/a)	-	-	n/a		Flaherty *et al.* (2011)
	-	1.3 µM (T)	-	n/a	n/a	border-line		Kenny *et al.* (2012)
		-	4.11 µM (P)	-	-	no		Wang *et al.* (2014)
	-	-	-	-	-	likely	~33% direct and ~50% NADPH- and time-dependent inhibition at 10 µM	Present study
Sunitinib (Sutent)	-	-	>150 µM (M), 54 µM (T), 9-28 µM (M)	-	-	n/a		http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021938_S000_Sutent_BioPharmR.pdf
	-	4.9 µM (T)	-	31.5 µM (M)	0.020 1/min (M)	yes		Kenny *et al.* (2012)
	-	-	20.35 µM (P)	-	-	likely	Increased inhibition after preincubation with NADPH at 10 µM, as compared to no preincubation	Wang *et al.* (2014)
	-	-	-	-	-	possibly	~50% direct and ~60% NADPH- and time-dependent inhibition at 55 µM	Present study
Vandetanib (Caprelsa)	-	-	-	-	-	no	At 100 µg/ml (210 µM) vandetanib inhibited marker reactions 0-22%	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022405Orig1s000PharmR.pdf
	-	-	-	-	-	no	No direct or TDI at 180 µM	Present study

Abbreviations: At, atorvastatin; F, felodipine; M, midazolam; N, nifedipine; n/a, not available; P, paclitaxel (formation of p-3'-hydroxypaclitaxel); T, testosterone; TDI, time-dependent inhibition; TDI IC_{50}, IC_{50} following preincubation with NADPH of the inhibitor.
[a] Unclear whether the study was performed using HLM or recombinant enzymes.

Supplementary Table S5. Examples of CYP2C8- and CYP3A-mediated interactions with the protein kinase inhibitors tested in the present study as perpetrator drugs.

Perpetrator kinase inhibitor	Kinase inhibitor dose	Victim drug	Victim dose	Observed AUC ratio of victim	References
Axitinib	5 mg BID	Docetaxel	100 mg/m^2 every 3 weeks	1.55	Martin *et al.* (2012)
		Paclitaxel	90 mg/m^2 weekly	1.04	
Crizotinib	250 mg BID	Midazolam	2 mg, SD	3.65	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf
Dasatinib	100 mg, SD	Simvastatin	80 mg, SD	1.20	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021986s000_Sprycel__ClinPharmR.pdf
Erlotinib	150 mg, OD	Midazolam	7.5 mg, SD	0.66-0.83	Calvert *et al.* (2014)
Nilotinib	200 or 400 mg, OD or 400 mg BID	Imatinib	400 mg, BID	1.0-1.4	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022068s000_ClinPharmR.pdf, Demetri *et al.* (2009)
	600 mg, SD	Midazolam	4 mg, SD	1.3	
Pazopanib	800 mg, OD	Midazolam	3 mg, SD	1.35	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_ClinPharmR.pdf, Goh *et al.* (2010)
	800 mg, OD	Paxlitaxel	80 mg/m^2 weekly	1.26	
Sorafenib	200 or 400 mg, BID	Docetaxel	75 or 100 mg/m^2 every 3 weeks	1.1-1.8	http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021923_s000_Nexavar_BioPharmR.pdf, Flaherty *et al.* (2011)
	400 mg, BID	Midazolam	2 mg, SD	0.85	
Sunitinib	50 or 100 mg daily	Docetaxel	60 or 75 mg/m^2 every 3 weeks	1.1-1.4	de Jonge *et al.* (2011)
	25 or 37.5 mg daily	Paclitaxel	90 mg/m^2 weekly	1.2	Kozloff *et al.* (2010)

Abbreviations: BID, twice daily; n/a, not available; OD, once daily: SD, single dose.

Supplementary Table S6. Examples of CYP3A-mediated interactions with the protein kinase inhibitors tested in the present study as victim drugs.

Victim kinase inhibitor	Main CYP enzymes involved in metabolism	Kinase inhibitor dose	Perpetrator drug	Perpetrator dose	Observed AUC ratio of kinase inhibitor	References
Axitinib	CYP3A4/5, CYP1A2, CYP2C19	5 mg, SD 5 mg, SD	Ketoconazole Rifampin	400 mg, OD 600 mg, OD	2.1 0.21	http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202324lbl.pdf, Pithavala *et al.* (2012), Pithavala *et al.* (2010), Rugo *et al.* (2005)
Bosutinib	CYP3A4	100 mg, SD 500 mg, SD	Ketoconazole Rifampin	400 mg, OD 600 mg, OD	8.6 0.060	http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203341lbl.pdf, Abbas *et al.* (2011)
Crizotinib	CYP3A4/5	150 mg, SD 250 mg, SD	Ketoconazole Rifampin	200 mg, BID 600 mg, OD	3.2 0.18	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/202570s004lbl.pdf
Dasatinib	CYP3A4	20 mg, OD 100 mg, SD	Ketoconazole Rifampin	200 mg, BID 600 mg, OD	4.8 0.18	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021986s013lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021986s000_Sprycel__ClinPharmR.pdf, Johnson *et al.* (2010), Wang *et al.* (2008)
Erlotinib	CYP3A4, CYP1A2, CYP2C8	100 mg, SD 150 mg, SD	Ketoconazole Rifampin	200 mg BID 600 mg, OD	1.6 0.19-0.34	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021743s018lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-743_Tarceva_biopharmr.PDF, Rakhit *et al.* (2008), Hamilton *et al.* (2014), Ling *et al.* (2006)
Gefitinib	CYP3A4/5, CYP2D6, CYP1A1	500 mg, SD 250 or 500 mg, SD	Itraconazole Rifampin	200 mg, OD 600 mg, OD	1.61-1.78 0.17	Swaisland *et al.* (2005)
Lestaurtinib	CYP3A4, CYP1A2, CYP2B6	n/a	n/a	n/a	n/a	http://aml17.cardiff.ac.uk/files/aml17_protocolv2.pdf
Nilotinib	CYP3A4, (CYP2C8)	200 mg, SD 400 mg, SD	Ketoconazole Rifampin	400 mg, OD 600 mg, OD	3.1 0.20	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022068s014lbledt.pdf, Tanaka *et al.* (2011)
Pazopanib	CYP3A4, (CYP2C8)	400 mg, OD	Ketoconazole	400 mg, OD	1.66	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022465s014s015s016lbl.pdf, Tan *et al.* (2013)

Saracatinib	CYP3A4	n/a	n/a	n/a	n/a	Hannon *et al.* (2010)
Sorafenib	CYP3A4	50 mg, SD 400 mg, SD	Ketoconazole Rifampin	400 mg, OD 600 mg, OD	0.89 0.63	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021923s014lbl.pdf, Lathia *et al.* (2006)
Sunitinib	CYP3A4, (CYP2C8)	10 mg, SD 50 mg, SD	Ketoconazole Rifampin	400 mg, OD 600 mg, OD	1.6-1.8 0.21-0.25	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021938s024s025lbl.pdf, http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021938_S000_Sutent_BioPharmR.pdf
Vandetanib	CYP3A4	300 mg, SD 300 mg, SD	Itraconazole Rifampin	200 mg, OD 600 mg, OD	1.1 0.60	http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022405s004lbl.pdf, Martin *et al.* (2011)

Abbreviations: BID, twice daily; n/a, not available; OD, once daily: SD, single dose.

References

Abbas R, Hug BA, Leister C, Burns J, and Sonnichsen D (2011) Effect of ketoconazole on the pharmacokinetics of oral bosutinib in healthy subjects. *J Clin Pharmacol* 51**:**1721-1727.

Baselga J, Cervantes A, Martinelli E, Chirivella I, Hoekman K, Hurwitz HI, Jodrell DI, Hamberg P, Casado E, Elvin P, Swaisland A, Iacona R, and Tabernero J (2010) Phase I safety, pharmacokinetics, and inhibition of SRC activity study of saracatinib in patients with solid tumors. *Clin Cancer Res* 16**:**4876-4883.

Calvert H, Twelves C, Ranson M, Plummer R, Fettner S, Pantze M, Ling J, Hamilton M, Lum BL, and Rakhit A (2014) Effect of erlotinib on CYP3A activity, evaluated in vitro and by dual probes in patients with cancer. *Anticancer Drugs* doi: 10.1097/CAD.0000000000000099

Daud AI, Krishnamurthi SS, Saleh MN, Gitlitz BJ, Borad MJ, Gold PJ, Chiorean EG, Springett GM, Abbas R, Agarwal S, Bardy-Bouxin N, Hsyu PH, Leip E, Turnbull K, Zacharchuk C, and Messersmith WA (2012) Phase I study of bosutinib, a Src/Abl tyrosine kinase inhibitor, administered to patients with advanced solid tumors. *Clin Cancer Res* 18**:**1092-1100.

de Jonge MJ, Dumez H, Kitzen JJ, Beuselinck B, Verweij J, Courtney R, Battista A, Brega N, and Schoffski P (2011) Phase I safety and pharmacokinetic study of SU-014813 in combination with docetaxel in patients with advanced solid tumours. *Eur J Cancer* 47**:**1328-1335.

Demetri GD, Casali PG, Blay JY, von Mehren M, Morgan JA, Bertulli R, Ray-Coquard I, Cassier P, Davey M, Borghaei H, Pink D, Debiec-Rychter M, Cheung W, Bailey SM, Veronese ML, Reichardt A, Fumagalli E, and Reichardt P (2009) A phase I study of single-agent nilotinib or in combination with imatinib in patients with imatinib-resistant gastrointestinal stromal tumors. *Clin Cancer Res* 15**:**5910-5916.

Dong PP, Fang ZZ, Zhang YY, Ge GB, Mao YX, Zhu LL, Qu YQ, Li W, Wang LM, Liu CX, and Yang L (2011) Substrate-dependent modulation of the catalytic activity of CYP3A by erlotinib. *Acta Pharmacol Sin* 32**:**399-407.

Flaherty KT, Lathia C, Frye RF, Schuchter L, Redlinger M, Rosen M, and O'Dwyer PJ (2011) Interaction of sorafenib and cytochrome P450 isoenzymes in patients with advanced melanoma: a phase I/II pharmacokinetic interaction study. *Cancer Chemother Pharmacol* 68**:**1111-1118.

Fujita K, Ando Y, Narabayashi M, Miya T, Nagashima F, Yamamoto W, Kodama K, Araki K, Endo H, and Sasaki Y (2005) Gefitinib (Iressa) inhibits the CYP3A4-mediated formation of 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin but activates that of 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin from irinotecan. *Drug Metab Dispos* 33**:**1785-1790.

Goh BC, Reddy NJ, Dandamudi UB, Laubscher KH, Peckham T, Hodge JP, Suttle AB, Arumugham T, Xu Y, Xu CF, Lager J, Dar MM, and Lewis LD (2010) An evaluation of the drug interaction potential of pazopanib, an oral vascular endothelial growth factor receptor tyrosine

kinase inhibitor, using a modified Cooperstown 5+1 cocktail in patients with advanced solid tumors. *Clin Pharmacol Ther* 88**:**652-659.

Hamilton M, Wolf JL, Drolet DW, Fettner SH, Rakhit AK, Witt K, and Lum BL (2014) The effect of rifampicin, a prototypical CYP3A4 inducer, on erlotinib pharmacokinetics in healthy subjects. *Cancer Chemother Pharmacol* doi: 10.1007/s00280-014-2390-3.

Hannon RA, Clack G, Rimmer M, Swaisland A, Lockton JA, Finkelman RD, and Eastell R (2010) Effects of the Src kinase inhibitor saracatinib (AZD0530) on bone turnover in healthy men: A randomized, double-blind, placebo-controlled, multiple-ascending-dose phase I trial. *J Bone Miner Res* 25**:**463-471.

Johnson FM, Agrawal S, Burris H, Rosen L, Dhillon N, Hong D, Blackwood-Chirchir A, Luo FR, Sy O, Kaul S, and Chiappori AA (2010) Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. *Cancer* 116**:**1582-1591.

Kenny JR, Mukadam S, Zhang C, Tay S, Collins C, Galetin A, and Khojasteh SC (2012) Drug-drug interaction potential of marketed oncology drugs: in vitro assessment of time-dependent cytochrome P450 inhibition, reactive metabolite formation and drug-drug interaction prediction. *Pharm Res* 29**:**1960-1976.

Kim MJ, Lee JW, Oh KS, Choi CS, Kim KH, Han WS, Yoon CN, Chung ES, Kim DH, and Shin JG (2013) The tyrosine kinase inhibitor nilotinib selectively inhibits CYP2C8 activities in human liver microsomes. *Drug Metab Pharmacokinet* 28**:**462-467.

Kozloff M, Chuang E, Starr A, Gowland PA, Cataruozolo PE, Collier M, Verkh L, Huang X, Kern KA, and Miller K (2010) An exploratory study of sunitinib plus paclitaxel as first-line treatment for patients with advanced breast cancer. *Ann Oncol* 21**:**1436-1441.

Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, and Sundaresan P (2006) Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. *Cancer Chemother Pharmacol* 57**:**685-692.

Li J, Zhao M, He P, Hidalgo M, and Baker SD (2007) Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. *Clin Cancer Res* 13**:**3731-3737.

Li X, He Y, Ruiz CH, Koenig M, Cameron MD, and Vojkovsky T (2009) Characterization of dasatinib and its structural analogs as CYP3A4 mechanism-based inactivators and the proposed bioactivation pathways. *Drug Metab Dispos* 37**:**1242-1250.

Li X, Kamenecka TM, and Cameron MD (2010) Cytochrome P450-mediated bioactivation of the epidermal growth factor receptor inhibitor erlotinib to a reactive electrophile. *Drug Metab Dispos* 38**:**1238-1245.

Ling J, Johnson KA, Miao Z, Rakhit A, Pantze MP, Hamilton M, Lum BL, and Prakash C (2006) Metabolism and excretion of erlotinib, a small molecule inhibitor of epidermal growth factor receptor tyrosine kinase, in healthy male volunteers. *Drug Metab Dispos* 34**:**420-426.

Mao J, Johnson TR, Shen Z, and Yamazaki S (2013) Prediction of crizotinib-midazolam interaction using the Simcyp population-based simulator: comparison of CYP3A time-dependent inhibition between human liver microsomes versus hepatocytes. *Drug Metab Dispos* 41**:**343-352.

Martin LP, Kozloff MF, Herbst RS, Samuel TA, Kim S, Rosbrook B, Tortorici M, Chen Y, Tarazi J, Olszanski AJ, Rado T, Starr A, and Cohen RB (2012) Phase I study of axitinib combined with paclitaxel, docetaxel or capecitabine in patients with advanced solid tumours. *Br J Cancer* 107**:**1268-1276.

Martin P, Oliver S, Robertson J, Kennedy SJ, Read J, and Duvauchelle T (2011) Pharmacokinetic drug interactions with vandetanib during coadministration with rifampicin or itraconazole. *Drugs R D* 11**:**37-51.

Pithavala YK, Tong W, Mount J, Rahavendran SV, Garrett M, Hee B, Selaru P, Sarapa N, and Klamerus KJ (2012) Effect of ketoconazole on the pharmacokinetics of axitinib in healthy volunteers. *Invest New Drugs* 30**:**273-281.

Pithavala YK, Tortorici M, Toh M, Garrett M, Hee B, Kuruganti U, Ni G, and Klamerus KJ (2010) Effect of rifampin on the pharmacokinetics of Axitinib (AG-013736) in Japanese and Caucasian healthy volunteers. *Cancer Chemother Pharmacol* 65**:**563-570.

Rakhit A, Pantze MP, Fettner S, Jones HM, Charoin JE, Riek M, Lum BL, and Hamilton M (2008) The effects of CYP3A4 inhibition on erlotinib pharmacokinetics: computer-based simulation (SimCYP) predicts in vivo metabolic inhibition. *Eur J Clin Pharmacol* 64**:**31-41.

Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM, Pithavala YK, Reich SD, Freddo JL, and Wilding G (2005) Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: Pharmacokinetic and clinical results. *J Clin Oncol* 23**:**5474-5483.

Schaiquevic P, Panetta JC, Throm S, Daw NC, Geyer JR, Furman WL, and Stewart CF (2008) Population pharmacokinetic (PK) analysis of gefitinib in pediatric cancer patients. *J Clin Oncol (Meeting Abstracts)* 26**:**Abstract no 2523.

Swaisland HC, Ranson M, Smith RP, Leadbetter J, Laight A, McKillop D, and Wild MJ (2005) Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. *Clin Pharmacokinet* 44**:**1067-1081.

Tan AR, Gibbon DG, Stein MN, Lindquist D, Edenfield JW, Martin JC, Gregory C, Suttle AB, Tada H, Botbyl J, and Stephenson JJ (2013) Effects of ketoconazole and esomeprazole on the pharmacokinetics of pazopanib in patients with solid tumors. *Cancer Chemother Pharmacol* 71**:**1635-1643.

Tanaka C, Yin OQ, Smith T, Sethuraman V, Grouss K, Galitz L, Harrell R, and Schran H (2011) Effects of rifampin and ketoconazole on the pharmacokinetics of nilotinib in healthy participants. *J Clin Pharmacol* 51**:**75-83.

Tou WI and Chen CY (2012) In silico investigation of potential SRC kinase ligands from traditional Chinese medicine. *PLoS One* 7**:**e33728.

van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Beijnen JH, and Schinkel AH (2009) Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *Drug Metab Dispos* 37**:**2305-2313.

Wang LF, Christopher LJ, Cui DH, Li WY, Iyer R, Humphreys WG, and Zhang DL (2008) Identification of the human enzymes involved in the oxidative metabolism of dasatinib: An effective approach for determining metabolite formation kinetics. *Drug Metab Dispos* 36**:**1828-1839.

Wang Y, Wang M, Qi H, Pan P, Hou T, Li J, He G, and Zhang H (2014) Pathway-dependent inhibition of paclitaxel hydroxylation by kinase inhibitors and assessment of drug-drug interaction potentials. *Drug Metab Dispos* doi: 10.1124/dmd.113.053793