https://doi.org/10.1016/j.cbi.2021.109401

The *N*-oxide metabolite of sorafenib inhibits hepatic CYP2C8

Pramod C. Nair[3], Tina B. Gillani,[1] Tristan Rawling,[2] and Michael Murray,[1,4]

[1]Pharmacogenomics and Drug Development Group, Discipline of Pharmacology, School of Medical Sciences, Sydney Medical School, University of Sydney, NSW 2006, AUSTRALIA, [2]School of Mathematical and Physical Sciences, Faculty of Science, University of Technology Sydney, Ultimo, New South Wales, 2007, AUSTRALIA, and [3]Department of Clinical Pharmacology and Flinders Centre for Innovation in Cancer, College of Medicine and Public Health, Flinders University, Bedford Park, SA 5042, AUSTRALIA

[4]Address for correspondence: Dr Michael Murray,
Discipline of Pharmacology,
University of Sydney,
NSW 2006, AUSTRALIA
Tel: (61-2)-9036-3259
Fax: (61-2)-9351-4447
email: michael.murray@sydney.edu.au

Running title: sorafenib *N*-oxide and CYP2C8 inhibition

Abstract

The multikinase inhibitor sorafenib (SOR) is a frontline agent in the treatment of hepatocellular and renal cancers. In recent clinical studies SOR has been evaluated increasingly in combination with other oncology agents, such as paclitaxel. However, the use of such combinations could increase the likelihood of pharmacokinetic drug-drug interactions (DDIs) and adverse events. It has been reported that SOR may inhibit a number of human drug oxidation pathways mediated by multiple CYPs. Oxidative biotransformation of SOR generates the pharmacologically active *N*-oxide metabolite (SNO) that has been shown to accumulate in the serum of some individuals who have been treated with the drug. Recent evidence has suggested that the metabolite SNO is more effective than the parent drug as an inhibitor of some CYP-mediated drug oxidations. Molecular docking studies have shown that SNO is associated with an increase in the binding interactions with active site amino acid residues in these enzymes. SOR has been implicated as an inhibitor of CYP2C8, which is an important catalyst in the oxidative elimination of oncology drugs such as paclitaxel and imatinib; inhibition is potentiated by NADPH-dependent biotransformation of SOR. The present study evaluated the potential contribution of SNO to the inhibition of CYP2C8 and the closely related enzyme CYP2C9. The principal finding to emerge was that SNO was ~2-fold more effective than SOR as an inhibitor of CYP2C8-mediated paclitaxel 6α-hydroxylation in human liver. Both SOR and SNO interacted with active site residues in the catalytic center of CYP2C8; there were four additional hydrogen and halogen bonding interactions involving SNO. In contrast, the binding of SOR and SNO in the active site of CYP2C9 and the capacity to inhibit microsomal losartan oxidation were similar. These findings suggest that SNO has the potential to contribute to pharmacokinetic interactions between SOR and drugs that are substrates for CYP2C8, perhaps in those individuals in whom SNO accumulates.

Keywords: sorafenib, sorafenib *N*-oxide, CYP2C8 inhibition, paclitaxel hydroxylation, metabolite inhibition, molecular docking

INTRODUCTION

The kinase inhibitor sorafenib (SOR) is used to treat patients with cancers of the liver and kidney (1,2). Although better tolerated than conventional oncology drugs, some patients experience toxicity with SOR that necessitates dosage modifications (1-3). Hepatic cytochrome P450 (CYP) 3A4 oxidizes SOR to its active *N*-oxide metabolite (SNO; Fig. 1) (4,5). However, low overall rates of SOR clearance may promote the accumulation of SOR and the metabolite SNO during therapy (3,6-9). SOR is used in combination with other anticancer agents to optimise treatment. However, such combinations may increase the incidence of adverse effects, possibly attributable in part to pharmacokinetic drug-drug interactions (DDIs) due to impaired drug clearance by CYPs.

SNO formation varies between patients due to individual differences in CYP3A4 activity (8). We found recently that CYPs 3A4 and 2D6 were inhibited more effectively by SNO than SOR because the binding of SNO within the active centers of the enzymes was more effective (10,11). These findings suggest that patients that produce more SNO may be at greater risk of pharmacokinetic DDIs. SOR also inhibits other CYPs, including CYP2C8 (12). Importantly, CYP2C8 inhibition was more pronounced following NADPH-dependent biotransformation of the drug; to our knowledge the potential contribution of major SOR metabolites like SNO has not been assessed to date. Several clinical studies have tested the combination of SOR with oncology drugs that are also CYP2C8 substrates for improved efficacy against a number of cancers. Thus, the clinical activity of paclitaxel was enhanced when combined with SOR and carboplatin in patients with ovarian, fallopian tube or peritoneal cancer and with paclitaxel/cisplatin/gemcitabine in patients with advanced urothelial cancer (13,14). In contrast, the combination did not appear to improve treatment of patients with locally advanced or metastatic HER2-negative breast cancer (15). However, it is noteworthy that the grade and frequencies of adverse events were greater with these combinations than with paclitaxel alone (13-15).

The present study assessed the capacities of SOR and SNO to inhibit CYP2C8-mediated paclitaxel oxidation in human liver microsomes. The major finding was that the metabolite SNO was more effective than to the parent drug against the activity. In docking studies, SNO interacted more effectively than SOR with key amino acid residues in the catalytic site of CYP2C8. We also assessed the capacity of SOR and SNO to inhibit the closely related CYP2C9 but their activities were similar. These findings suggest that SNO has the potential to contribute to the inhibition of CYP2C8 by SOR.

MATERIALS AND METHODS

Chemicals and drugs

SOR (4-[4-([4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino)phenoxy]-*N*-methylpyridine-2-carboxamide) and SNO were prepared as previously described (10). Paclitaxel, its 6α-hydroxy-metabolite, other CYP substrates and biochemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). The active carboxylic acid metabolite of losartan (E-3174) was from SynFine Research Inc (Richmond Hills, ON, Canada), 1'-hydroxymidazolam was obtained from Cerilliant (Round Rock, TX) and other CYP metabolites were from Sigma-Aldrich. Microsomal fractions from insect cells that over-expressed CYP2C8 were purchased from BD Biosciences (Supersomes; North Ryde, NSW, Australia). HPLC grade solvents and analytical reagents were obtained from LabScan (Lomb Scientific, Taren Point, NSW, Australia) or Ajax Chemicals (Sydney, NSW, Australia).

Preparation of human liver microsomal fractions

The Human Ethics committee of the University of Sydney approved the present work in accordance with the World Medical Association guidelines. Liver tissue was obtained from the normal margin during surgical liver resection and was provided by the Australian and Queensland Liver Transplantation Programs (located at Royal Prince Alfred Hospital, Sydney, NSW, and Princess Alexandria Hospital, Brisbane, Queensland, respectively). At collection tissue was immediately placed in Viaspan solution (DuPont, Wilmington, DE, USA) and then transferred to liquid nitrogen. Microsomal fractions were prepared from three individual donors by ultracentrifugation (16). Liver microsomal protein was quantified by standard methods (17).

CYP substrate oxidation assays in human hepatic microsomal fractions

Paclitaxel 6α-hydroxylation was used to assess microsomal CYP2C8 activity (18). Incubations (0.2 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 25 μM paclitaxel and 0.15 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 90 min with methyl *tert*-butyl ether, the internal standard cephalomanine was added and extracts were dried under nitrogen.

Extracts were applied to an Alltech® Alltima™C18, 5μm 150 × 2.1 mm column (Grace Davison Discovery Sciences; Baulkham Hills, NSW, Australia) coupled to a Thermo Scientific TSQ Quantum Access Max liquid chromatography-mass spectrometry (LC-

MS/MS) system (San Jose, CA, USA), operating in positive electro-spray ionization mode. The mobile phase was methanol-water (50:50, v/v) containing 0.1% acetic acid and the flow rate was 0.3 mL/min; the data were analysed using Xcalibur 1.2 (Thermo Fisher, Waltham, MA).

Losartan oxidation was used to assess microsomal CYP2C9 activity (19). Incubations (0.25 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 25 μM losartan and 0.05 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 15 min with methyl *tert*-butyl ether and the internal standard phenacetin was added. Solid phase extraction was carried out on Waters Oasis HLB cartridges coupled to a Supelco Visiprep TM 24 system. Cartridges were washed twice with water (1 mL) and 10% methanol in water (1 mL) and were then eluted with methanol. Samples were subjected to LC-MS/MS as described above. The mobile phase was acetonitrile-water (50:50, v/v) containing 0.1% formic acid and the flow rate was 0.3 mL/min.

7-Ethoxyresorufin *O*-deethylation activity was used to assess microsomal CYP1A2 activity (20). Incubations (2 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 12.5 mM 7-ethoxyresorufin and 0.2 mg protein and were initiated with NADPH (1 mM final). Product (resorufin) formation was monitored by continuous spectrofluorometry at the excitation/emission wavelength pair 560/580 nm.

Dextromethorphan *O*-demethylation was used to assess microsomal CYP2D6 activity (19). Incubations (0.25 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 16 μM dextromethorphan and 0.15 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 30 min with methyl *tert*-butyl ether and the internal standard phenacetin was added. Solid phase extraction and LC-MS/MS analysis was conducted as described for losartan oxidation.

N-Nitrosodimethylamine *N*-demethylation activity was used to assess microsomal CYP2E1 activity (21). Incubations (1 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 4 mM *N*-nitrosodimethylamine and 2.5 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 20 min by the addition of 0.6 M trichloracetic acid and centrifuged at 1,000xg for 5 min. Ammonium acetate and acetylacetone were added (final concentrations 1.6 M and 16 mM, respectively) to 0.6 mL aliquots from incubations, heated to 37°C for 30 min and quantified spectrophotometrically at a wavelength of 412 nm (22).

Midazolam 1'-hydroxylation was used to assess microsomal CYP3A4 activity (23). Incubations (0.5 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 5 μM

midazolam and 0.1 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 5 min with acetonitrile and the internal standard phenacetin was added. Solid phase extraction and LC-MS/MS analysis was conducted as described for losartan oxidation.

Testosterone 6β-hydroxylation activity was also used to assess microsomal CYP3A4 activity (24). Incubations (0.4 mL in 0.1 M potassium phosphate buffer, pH 7.4, 37°C) contained 50 μM ^{14}C-testosterone (0.18 μCi) and 0.15 mg protein and were initiated with NADPH (1 mM final). Reactions were terminated after 2.5 min by the addition of ice-cold chloroform and applied to thin-layer chromatography plates, subjected to autoradiography and quantified by scintillation spectrometry. Linearity of product formation was established for all CYP reactions.

Inhibition of CYP2C-dependent paclitaxel and losartan oxidations by SOR and SNO

The capacity of SOR and SNO (10, 30 and 50 μM) to inhibit CYP2C8-mediated paclitaxel 6α-hydroxylation and CYP2C9-mediated losartan oxidation was assessed initially in human liver microsomes and cDNA-over-expressed Supersomes. Human liver microsomes were also used in kinetic studies of paclitaxel 6α-hydroxylation (6.25-50 μM concentration range). The kinetics of 6α-hydroxypaclitaxel formation (V) against paclitaxel (S) concentration were analysed by non-linear regression with r^2 values determined for all regression lines (GraphPad Prism 5; San Diego, CA). Lineweaver-Burk and Dixon plots and corresponding replots were constructed to identify the mode of inhibition (25). K_i values were derived from x-intercepts of the Lineweaver-Burk slope replots. Total drug concentrations were used in the present analyses because this has been found to improve DDI predictions with hydrophobic drugs like paclitaxel and SOR that may accumulate in liver (26).

Computational studies of the docking of SOR and SNO into the active sites of CYP2C8 and CYP2C9

Molecular docking was used to evaluate detailed interactions between CYP2C enzymes and SOR and SNO. The X-ray crystal structures of CYP2C8 (2VNO) and CYP2C9 (1R9O) were used. Unresolved residues in the 1R9O structure (38-42; 214-219) were built using the modloop program (https://modbase.compbio.ucsf.edu/modloop/) (27).

Protein structures were prepared by including H-atoms and Kollman all atom charges using the BioPolymer module of SYBYL (version X-2.1, Certara, Princeton, NJ, USA).

Unresolved residues in the N- and C-termini of X-ray structures, which are distant from the catalytic site, were excluded from the analysis.

Three-dimensional coordinates of SOR and SNO in sdf format were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/), and molecular modeling was achieved using SYBYL, installed on a Red Hat Linux 6.9 OS workstation. After the assignment of Gasteiger–Huckel partial atomic charges (28), energy minimization was performed using Powell's conjugate gradient method in conjunction with a Tripos 5.2 force field (29,30). A minimum energy difference of 0.001 kcal/mol was set as the convergence criterion.

Molecular docking experiments were conducted using the Surflex-Dock docking suite (31) as previously reported (11,32). The resulting binding poses were ranked according to the total score (SYBYL Surflex-Dock). The docked complexes were analysed by the protein-ligand interaction profiler server (https://projects.biotec.tu-dresden.de/plip-web/plip/index)

Statistics

Data are expressed throughout as means±SEM of individual estimates as indicated.

RESULTS

Human liver donors and microsomal CYP substrate oxidations

Tissue from three liver donors (designated HL5, HL7 and HL9) was available for this study. For two of the donors some demographic information and drug history was available (HL7: male 53 years and HL9: male 37 years; both donors received spironolactone). Several microsomal CYP substrate oxidation activities were measured in the livers. Thus, CYP2C8-mediated paclitaxel 6α-hydroxylation varied over a 5.8-fold range (11-64 pmol 6α-hydroxypaclitaxel formation/mg protein/min) and CYP2C9-mediated losartan oxidation activity varied over a 3.3-fold range (25-83 pmol E-3174 formation/mg protein/min) across the three microsomal fractions (Table I). Rates of CYP1A2-mediated 7-ethoxyresorufin *O*-deethylation, CYP2D6-mediated dextromethorphan O-demethylation, CYP2E1-mediated *N*-nitrosodimethylamine *N*-demethylation and CYP3A4-mediated midazolam 1'-hydroxylation and testosterone 6β-hydroxylation varied over 4.2-, 2.2-, 31-, 1.9- and 16-fold ranges, respectively (Table I).

SOR and its major oxidized metabolite SNO as inhibitors of microsomal CYP2C8 and CYP2C9 activities

In the three hepatic microsomal fractions SOR elicited concentration-dependent decreases in CYP2C8-mediated paclitaxel 6α-hydroxylation; however, more pronounced decreases were elicited by SNO at each concentration (Fig 2a). In confirmation of this finding, IC_{50}s for SNO and SOR against cDNA-expressed human CYP2C8 were 22 μM and 38 μM, respectively (not shown). In contrast, the inhibitory activities of SOR and SNO were similar against CYP2C9-mediated losartan oxidation (Fig 2b).

To pursue the differential susceptibility of CYP2C8 activity to SOR and SNO kinetic studies were undertaken. The data were fitted to alternate models of inhibition (GraphPad Prism 5). In these studies the K_m value for microsomal paclitaxel 6α-hydroxylation was 34 ± 7 μM (Fig 3a), while the V_{max} values in HL5, HL7 and HL9 were 19, 20 and 96 pmol/min/mg protein. The optimal fit was obtained for linear-mixed inhibition kinetics, according to the Henri-Michaelis-Menten equation:

$$\frac{V}{V_{max}} = \frac{S}{K_m\left(1+\frac{I}{K_i}\right) + S\left(1+\frac{I}{aK_i}\right)}$$

From Lineweaver-Burk plots (Fig 3b, 3c) and the corresponding Lineweaver-Burk slope replots (Fig 3d) the inhibition of CYP2C8 activity by SOR and SNO was linear-mixed

(25). K_is were obtained from Lineweaver-Burk slope replot x-intercepts (Fig 3d). For SNO and SOR K_is were 12±2 μM and 36±2 μM, respectively. The parameter 'a' describes the increase in the equilibrium constants K_m and K_i by inhibitor and substrate, respectively (17; Fig. 4); a values were similar for both SNO and SOR (4.5±2.0 and 3.5±2.0, respectively).

Molecular docking of SOR and SNO with CYP2C8

Molecular docking of SOR and SNO was undertaken using the CYP2C8 X-ray crystal structure (2VN0) that was resolved in the presence of the bound inhibitor troglitazone (Fig 5a, 5b). Although similar binding modes were evident with the two molecules, SNO was positioned more closely than SOR to the iron atom of the heme prosthetic group in the enzyme (5.1 Å compared with 6 Å; Fig 5a, 5b). The oxygen atom of the *N*-oxide group in SNO was located 4.4 Å from the heme Fe atom.

Several interactions with CYP2C8 active site residues were common to the binding of both SOR and SNO. Thus, Ser 103 formed a hydrogen bond with a urea nitrogen in both molecules, and hydrophobic interactions were noted between the CF_3-substituted aromatic ring and the residues Asn 217 and Pro 367 (Table II). A further hydrophobic interaction between the central aromatic ring and Val 306 was noted. Two further residues were involved in binding of SOR and SNO but the nature of the interactions differed between the ligands. The side chain hydroxyl in Ser 100 was involved in a hydrogen bond with a urea nitogen atom in SOR but in the case of SNO this bond was with the urea oxygen. Ile 113 was involved in a hydrophobic interaction with the pyridine ring in SOR and the central aromatic system in SNO (Table II).

Two further interactions were noted between SOR and CYP2C8 active site residues: a halogen bond between Gln 214 (acceptor) and a fluorine atom in the CF_3-substituted aromatic ring (donor) and a hydrophobic interaction between Ile 476 and the central aromatic ring. In contrast, several additional bonding interactions were noted with SNO. In particular, there were three hydrogen bonds between the backbone oxygen in Gly 98 and a urea nitrogen, and the pyridine ring amide nitrogen substituent and both Val 296 and Thr 301. Asn 218 was also involved in a halogen bond with the aromatic chloro substituent in SNO and there were further hydrophobic interactions between Val 296 and Ala 297 and the NO moiety (Fig 5b, Table II). Overall, the larger number of active site interactions involving SNO and its closer proximity to the CYP2C8 heme was associated with an increase in the estimated binding

affinity from docking studies that is consistent with the observed findings from inhibition studies (Table III).

Comparative docking studies of SOR/SNO with CYP2C9 (1R9O structure) were also undertaken (Fig 6a, 6b). In the case of this enzyme most active site interactions were common to both molecules. Thus, common hydrogen bonds from the nitrogen and oxygen atoms in the amide substituent on the picolinamide system were noted with Ser 209 and Asn 474, and to the pyridine nitrogen and Thr 304. Hydrophobic and π-π stacking interactions involving Phe 110, Phe 114 and Phe 476 with the CF_3-substituted aromatic systems were also common to both SOR and SNO. A further hydrophobic interaction was identified between Leu 362 and the central aromatic ring in SOR (Fig 6a). Leu 208 also participated in binding but the nature of the interaction differed between SOR (a halogen bond with the fluorine atom in the CF_3-substituted aromatic system) and SNO (a hydrogen bond to a urea nitrogen atom; Fig 6a, 6b). Similarly, Leu 361 participated in hydrophobic interactions with the pyridine system in SOR and the pyridine *N*-oxide in SNO. Several interactions were noted with SNO that were not evident with SOR (Fig 6b; Table II). These included a halogen bond between Leu 102 and the chlorine atom in the aromatic system and hydrophobic interactions involving Ile 205, Leu 208, Glu 300 and Thr 301 and the aromatic systems in SNO. Taken together, however, these differences in binding behaviour between SOR/SNO and CYP2C9 were fewer that the corresponding differences in CYP2C8 active site interactions. Overall, the similarities in the binding modes of SOR/SNO with CYP2C9 are consistent with the similar inhibition effectiveness against losartan oxidation (Fig 2b).

DISCUSSION

It has been reported that the kinase inhibitor SOR inhibits human CYP2C8 and that the extent of inhibition is enhanced following biotransformation in NADPH-fortified hepatic microsomes (12). In the present study SNO - the major pharmacologically active metabolite of SOR - was found to be more effective than SOR as an inhibitor of CYP2C8-mediated paclitaxel 6α-hydroxylation in human liver. This is similar to previous findings in which SNO was found to be more potent than the parent drug as an inhibitor of CYP3A4 and CYP2D6 (10, 11). Kinetic studies in human liver microsomes were undertaken to assess the inhibition of CYP2C8 in greater detail. The K_i for SNO against microsomal CYP2C8-mediated paclitaxel 6α-hydroxylation (12±2 μM) was three-fold lower than that for SOR (36±2 μM), indicating greater affinity for the enzyme. Because the K_m for paclitaxel was ~34 μM, CYP2C8 has also an approximate 2.3-fold greater affinity for SNO than for paclitaxel. In contrast, the affinity of CYP2C8 for paclitaxel and SOR was similar, which is consistent with the lower inhibition potency of SOR. Accordingly, the present findings suggest that SNO has the potential to contribute to CYP2C8 inhibition. In comparative studies, we also assessed the capacity of SOR and SNO to inhibit CYP2C9, which shares 85% amino acid sequence identity with CYP2C8 (33). However, unlike CYP2C8, CYP2C9 was inhibited to a similar extent by both SOR and SNO.

To understand the inhibition findings molecular docking of SOR and SNO was undertaken using X-ray crystal structures of CYP2C8 and CYP2C9. In the case of CYP2C8 the *N*-oxide oxygen atom of SNO was bound 4.4 Å from the heme group that mediates oxygen coordination and activation in preparation for substrate oxidation. The distance between the pyridine nitrogen and the heme iron in the SNO-docked structures was 5.1 Å, compared with 6 Å in the SOR-docked enzyme. There were eight interactions between SOR and amino acid residues in the active center of CYP2C8. Six of these were common to SNO binding, including two hydrogen bonds and four hydrophobic interactions. Two further interactions with separate residues were noted with SOR only, including a halogen bond between a fluorine atom (donor) from the CF_3 aromatic substituent and the side chain oxygen (acceptor) of Gln 214, and a hydrophobic interaction involving Ile 476. However, the number of interactions for SNO in the structure of CYP2C8 was greater than for SOR. There were four additional hydrogen and halogen bonds and two further hydrophobic interactions with active site residues that were not observed with SOR. These higher energy hydrogen and

315 halogen bonds in particular are likely to contribute significantly to the efficiency of SNO
316 binding in the CYP2C8 structure.

317 Docking of SOR and SNO with the CYP2C8 X-ray crystal structure (2VN0) revealed
318 interactions with residues located in the active site cavity of the enzyme (34). Thus, Ser 100
319 and Ser 103 in helix B′ formed hydrogen bonds with the urea moieties in SNO/SOR. These
320 residues have been implicated previously in hydrogen bonding to the carboxylate substituents
321 of montelukast and troglitazone (35). Similarly, Gly-98 in the B-B'-loop was implicated in
322 hydrogen bonding to the substrate all-*trans* retinoic acid (35). In addition, Gln 214 in helix F'
323 was involved in halogen bonding with SOR, while Asn 218 in helix F' and Val 296 and Thr
324 301 were involved in halogen/hydrogen bonding to SNO.

325 Docking of SOR/SNO in the CYP2C9 (1R9O) structure was also undertaken.
326 Interactions with eight residues in the active center of CYP2C9 were common to both
327 molecules, including four hydrogen or halogen bond and four hydrophobic and/or π-π
328 stacking interactions. SOR formed an additional hydrophobic interaction with Leu 361 that
329 was not evident in the SNO-CYP2C8 structure. SNO participated in an additional halogen
330 bonding interaction with Leu 102 in the B-C-loop that is part of putative substrate recognition
331 sequence (SRS)-2, and the chlorine atom in the aromatic system and hydrophobic interactions
332 involving Ile 205, Leu 208, Glu 300 and Thr 301. Some of the residues implicated in
333 SOR/SNO docking interactions are also involved in the binding of other molecules in the
334 CYP2C9 active site, including Phe 100 and Leu 361 and tolbutamide binding (36), Phe 114
335 in SRS-1 is important for S -warfarin, diclofenac and flurbiprofen metabolism (37-39). Phe
336 114, Leu362, and Phe476 form important interactions with inhibitors (40).

337 The binding pocket in CYP2C8 is larger (740 $Å^3$) than that in CYP2C9 (510 $Å^3$),
338 which is consistent with the observation that CYP2C8 can efficiently oxidize relatively large
339 substrates like paclitaxel (41). In CYP2C8 access to the active site is facilitated by Ile102,
340 Ser114, Leu208, Val366, and Ile476 because these residues have smaller side chains than the
341 corresponding residues in CYP2C9 (41). The CYP2C8 active site is also more polar than
342 CYP2C9 because of Asn99 and Ser114. The number of high energy binding interactions with
343 CYP2C8 (halogen and hydrogen bonds) was greater for SNO (six) than for SOR (three). In
344 comparison, there were four and five interactions of this type for the interaction of CYP2C9
345 with SOR and SNO, respectively. Apparent binding affinities for interactions of the
346 molecules with CYP2C8/CYP2C9 were calculated, and were consistent with the observed

findings from inhibition studies (Table III). However, as suggested previously, these values should be interpreted cautiously (11).

SOR is being used increasingly in combination with other drugs in cancer chemotherapy but this increases the possibility of pharmacokinetic DDIs and adverse effects. Regimen containing SOR, carboplatin and the CYP2C8 substrate paclitaxel improved the response rate and progression free survival in patients with ovarian, fallopian tube, or primary peritoneal cancer, but increased toxicities (13). Further, SOR increased survival benefits with aclitaxel/cisplatin/gemcitabine and gemcitabine/cisplatin but also caused a relatively higher incidence of adverse events in patients with advanced urothelial cancer (14). In patients with locally advanced or metastatic HER2-negative breast cancer the paclitaxel-SOR combination was not superior to paclitaxel monotherapy and the safety and toxicity profile of the combination therapy was less favorable (15). In a completed trial (NCT00558636) the addition of SOR to a regimen of paclitaxel and carboplatin increased the incidence of serious adverse events in 91 non-small cell lung cancer patients from 6.8% to 23.4%. On the other hand, other studies have claimed that toxicity was manageable with dose reductions when SOR was added to paclitaxel-containing regimen (42, 43). In another study the combination did not increase adverse events but there was some evidence of increased paclitaxel exposure (AUC and C_{max} increased up to 1.55- and 2.21-fold of those after paclitaxel alone); however, the small sample size prevented statistical significance from being attained (44).

CYP2C8 is also important in the biotransformation of other drugs, including imatinib (18). An individual with chronic myloid leukemia and thyroid carcinoma received the imatinib/SOR combination that was initially well tolerated (45). However, after twenty-one months the patient died of sudden myocardial infarction. Separately it was *suggested that, even though* the dose-normalised serum concentrations of imatinib were 1.7-fold higher than expected, the drug combination was feasible (46). It remains a possibility, however, that increased imatinib exposure could have contributed to myocardial toxicity.

A number of drugs and xenobiotics generate metabolites that are more effective than the parent agents as CYP inhibitors. These include reactive metabolites generated during biotransformation of alkylamine, benzodioxole, thionosulfur and alkene/alkyne-containing chemicals (47-53). There is an increasing number of stable metabolites, including N-oxide metabolites, that have a greater propensity for CYP inhibition than their precursor chemicals (54-56). SOR pharmacokinetics are complex and inter-individual variation is extensive (6,7 OK). The systemic availabilities of SOR and SNO are increased in some patients after long-term therapy (4,6-9). Thus, the C_{max} of SNO reached 8.5 µM in the serum of one patient; this

is of similar magnitude to the K_i determined for CYP2C8 inhibition. Other studies found that SNO can attain serum concentrations of 1-5 μM in some patients (7, 57, 58). Accordingly, there is the potential that SOR may elicit pharmacokinetic DDIs in patients who produce high concentrations of SNO. CYP3A4 is the major catalyst of SOR oxidation (5), and the activity of the enzyme is subject to variation caused by genetic polymorphisms, exposure to drugs and other chemical inhibitors and inducers (54 OK), female sex and liver disease (54-61). The present study adds to the evidence that the N-oxide SOR metabolite SNO may contribute to the inhibition of multiple CYPs. It would now be of interest to evaluate these possibilities directly in clinical studies.

CONCLUSIONS

The present study suggests that SNO is more potent than SOR as an inhibitor of CYP2C8 but not the closely-related enzyme CYP2C9. In docking studies, SOR interacted with multiple amino acid residues in the active site of CYP2C8. Compared with SOR, SNO was involved in additional interactions of higher energy (halogen and hydrogen bonds), which is consistent with its greater inhibitory potency. The binding of SOR and SNO in the active center of CYP2C9 was similar. SOR continues to be evaluated in combination with other anti-cancer and adjunct treatments in patients. However, the drug is contra-indicated with a large number of agents and some clinical trials of such combinations have been terminated because of a high incidence of adverse effects. Whether this is due to pharmacokinetic DDIs has not been established. To complement the present study it would now be of interest to assess SNO production directly in patients who are receiving drug combinations containing SOR. Moreover, serum SNO monitoring during therapy may enable individual patients to be identified who might benefit from drug regimen modifications in order to avoid SOR-mediated DDIs.

CONFLICTS OF INTEREST

None

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from Cancer Council NSW (grants RG09-14 and IG11-33). PCN acknowledges the Flinders Centre for Innovation in Cancer (FCIC) and Flinders Medical Centre (FMC) Foundation for an Early Career Research Grant. The supply of the human liver samples used in this study by Dr J George is also gratefully acknowledged.

ABBREVIATIONS

AUC, area under the serum concentration versus time curve; C_{max}, maximal serum concentration; CYP, cytochrome P450; DDI, drug-drug interaction; SOR, sorafenib; SNO, sorafenib *N*-oxide.

REFERENCES

1. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. New Engl J Med. 2007;356:125-34. doi: 10.1056/NEJMoa060655.
2. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, et al. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Cancer Res. 2006;66:11851-8. doi**:** 10.1158/0008-5472.CAN-06-1377.
3. Awada A, Hendlisz A, Christensen O, Lathia CD, Bartholomeus S, Lebrun F, et al. Phase I trial to investigate the safety, pharmacokinetics and efficacy of sorafenib combined with docetaxel in patients with advanced refractory solid tumours. Eur J Cancer 2012;48:465-74. doi: 10.1016/j.ejca.2011.12.026.
4. Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, Sundaresan P. Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. Cancer Chemother Pharmacol. 2006;57:685-92. doi.org/10.1007/s00280-005-0068-6.
5. Ghassabian S, Rawling T, Zhou F, Doddareddy MR, Tattam BN, Hibbs DE, et al. Role of human CYP3A4 in the biotransformation of sorafenib to its major oxidized metabolites. Biochem Pharmacol. 2012;84:215-23. doi: 10.1016/j.bcp.2012.04.001.
6. Strumberg D, Clark JW, Awada A, Moore MJ, Richly H, Hendlisz A, et al. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. Oncologist 2007;12:426-37. doi: 10.1634/theoncologist.12-4-426.
7. Minami H, Kawada K, Ebi H, Kitagawa K, Kim YI, Araki K, et al. Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors. Cancer Sci. 2008;99:1492-8. doi: 10.1111/j.1349-7006.2008.00837.x.
8. Inaba H, Rubnitz JE, Coustan-Smith E, Li L, Furmanski BD, Mascara GP, et al. Phase I pharmacokinetic and pharmacodynamic study of the multikinase inhibitor sorafenib in combination with clofarabine and cytarabine in pediatric relapsed/refractory leukemia. J Clin Oncol. 2011;29:3293-300. doi: 10.1200/JCO.2011.34.7427.
9. Ferrario C, Strepponi I, Esfahani K, Charamis H, Langleben A, Scarpi E, et al. Phase I/II trial of sorafenib in combination with vinorelbine as first-line chemotherapy for metastatic breast cancer. PLoS ONE 2016;11:e0167906. doi: 10.1371/journal.pone.0167906.

10. Ghassabian S, Gillani TB, Rawling T, Crettol S, Nair PC, Murray M. Sorafenib N-oxide is an inhibitor of human hepatic CYP3A4. AAPS J. 2019; 21:15. doi. 10.1208/s12248-018-0262-1.
11. Murray M, Gillani TB, Rawling T, Nair PC. Inhibition of Hepatic CYP2D6 by the Active N-Oxide Metabolite of Sorafenib. AAPS J. 2019;21:107. doi: 10.1208/s12248-019-0374-2.
12. Wang Y, Wang M, Qi H, Pan P, Hou T, Li J, et al. Pathway-dependent inhibition of paclitaxel hydroxylation by kinase inhibitors and assessment of drug-drug interaction potentials. Drug Metab Dispos. 2014;42:782-95. doi: 10.1124/dmd.113.053793.
13. Schwandt A, von Gruenigen VE, Wenham RM, Frasure H, Eaton S, Fusco N, et al. Randomized phase II trial of sorafenib alone or in combination with carboplatin/paclitaxel in women with recurrent platinum sensitive epithelial ovarian, peritoneal, or fallopian tube cancer. Invest New Drugs. 2014;32:729-38. doi: 10.1007/s10637-014-0078-5.
14. Wang Y, Xu L, Meng X, Qin Z, Wang Y, Chen C, et al. Different chemotherapy regimens in the management of advanced or metastatic urothelial cancer: a Bayesian network meta-analysis of randomized controlled trials. Cell Physiol Biochem 2018;50:1-14. doi: org/10.1159/000493951.
15. Decker T, Overkamp F, Rosel S, Nusch A, Gohler T, Indorf M, et al. A randomized phase II study of paclitaxel alone versus paclitaxel plus sorafenib in second- and third-line treatment of patients with HER2-negative metastatic breast cancer (PASO). BMC Cancer 2017;17:499. doi: 10.1186/s12885-017-3492-1.
16. Murray M. Metabolite intermediate complexation of microsomal cytochrome P450 2C11 in male rat liver by nortriptyline. Mol Pharmacol. 1992;42:931-8.
17. M Murray. Participation of a cytochrome P450 enzyme from the 2C subfamily in progesterone 21-hydroxylation in sheep liver. J Steroid Biochem Mol Biol. 1992;43: 591-3. doi: 10.1016/0960-0760(92)90248-h.
18. Nebot N, Crettol S, d'Esposito F, Tattam B, Hibbs DE, Murray M. Participation of CYP2C8 and CYP3A4 in the *N*-demethylation of imatinib in human hepatic microsomes. Brit J Pharmacol. 2010;161: 1059-69. doi: 10.1111/j.1476-5381.2010.00946.x
19. Ghassabian S, Chetty M, Tattam BN, Glen J, Rahme J, Stankovic Z, et al. A high-throughput assay using liquid chromatography/tandem mass spectrometry for

simultaneous in vivo phenotyping of five major cytochrome P450 enzymes in patients. Ther Drug Monit. 2009;31:239-46. doi: 10.1097/FTD.0b013e318197e1bf.

20. Marcus CB, Murray M, Wilkinson CF. Spectral and inhibitory interactions of methylenedioxyphenyl and related compounds with purified isozymes of cytochrome P-450. Xenobiotica 1985;15:351-62. doi: 10.3109/00498258509045370.

21. Peng R, Tennant P. Lorr NA, Yang CS. Alterations of microsomal monooxygenase system and carcinogen metabolism by streptozotocin-induced diabetes in rats. Carcinogenesis 1983;4: 703-8. doi: 10.1093/carcin/4.6.703.

22. Murray M, Hetnarski K, Wilkinson CF. Selective inhibitory interactions of alkoxymethylenedioxybenzenes towards monooxygenase activity in rat hepatic microsomes. Xenobiotica 1985;15:369-79.

23. d'Esposito F, Tattam BN, Ramzan I, Murray M. A liquid chromatography/electrospray ionization mass spectrometry (LC/MS/MS) assay for the determination of irinotecan (CPT-11) and its two major metabolites in human liver microsomal incubations and human plasma samples. J Chromatogr B 2008;875:522-30. doi: 10.1016/j.jchromb.2008.10.011

24. Murray M, Zaluzny L, Dannan GA, Guengerich FP, Farrell GC. Altered regulation of cytochrome P-450 enzymes in choline-deficient cirrhotic male rat liver: Impaired regulation and activity of the male-specific androst-4-ene-17-dione 16α-hydroxylase, cytochrome P-450$_{UT\text{-}A}$, in hepatic cirrhosis. Mol Pharmacol. 1987;3:117-21.

25. Johnston RA, Rawling T, Chan T, Zhou F, Murray M. Selective inhibition of human solute carrier transporters by multikinase inhibitors. Drug Metab Dispos. 2014;42:1851-7. doi: 10.1124/dmd.114.059097.

26. von Moltke LL, Greenblatt DJ, Cotreau-Bibbo MM, Duan SX, Harmatz JS, Shader RI. Inhibition of desipramine hydroxylation in vitro by serotonin-reuptake-inhibitor antidepressants, and by quinidine and ketoconazole: a model system to predict drug interactions in vivo. J Pharmacol Exp Ther. 1994;268:1278-83.

27. Fiser A, Sali A. ModLoop: automated modeling of loops in protein structures. Bioinformatics. 2003; 19:2500-1. doi: 10.1093/bioinformatics/btg362

28. Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. Tetrahedron 1980;36:3219-28. doi.org/10.1016/0040-4020(80)80168-2.

519 29. Powell MJD. Restart procedures for the conjugate gradient method. Math Program.
520 1977;12:241-54. doi.org/10.1007/BF01593790.
521 30. Clark M, Cramer RD III, Van Opdenbosch N. Validation of the general purpose tripos
522 5.2 force field. J Comp Chem. 1989;10:982-1012. doi.org/10.1002/jcc.540100804.
523 31. Jain AN. Surflex: fully automatic flexible molecular docking using a molecular
524 similarity-based search engine. J Med Chem. 2003;46:499-511. doi:
525 10.1021/jm020406h.
526 32. Nair PC, McKinnon RA, Miners JO. Computational prediction of the site(s) of
527 metabolism and binding modes of protein kinase inhibitors metabolized by CYP3A4.
528 Drug Metab Dispos. 2019;47:616-31. doi: 10.1124/dmd.118.085167.
529 33. Boye SL, Kerdpin O, Elliott DJ, Miners JO, Kelly L. Optimizing bacterial expression
530 of catalytically active human cytochromes P450: comparison of CYP2C8 and CYP2C9.
531 Xenobiotica 2014;34: 49-60, 2004. doi: 10.1080/00498250310001636868.
532 34. Schoch GA, Yano JK, Wester MR, Griffin KJ, Stout CD, Johnson EF. Structure of
533 human microsomal cytochrome P450 2C8. Evidence for a peripheral fatty acid binding
534 site. J Biol Chem. 2004;279: 9497-503. doi: 10.1074/jbc.M312516200.
535 35. Schoch GA, Yano JK, Sansen S, Dansette PM, Stout CD, Johnson EF. Determinants of
536 Cytochrome P450 2C8 Substrate Binding. Structures of complexes with montelukast,
537 troglitazone, felodipine, and 9-*cis*-retinoic acid. J Biol Chem. 2008; 283: 17227-37. doi:
538 10.1074/jbc.M802180200
539 36. Payne VA, Chang YT, Loew GH. Homology modeling and substrate binding study of
540 human CYP2C18 and CYP2C19 enzymes. Proteins. 1999;37: 204-17. doi:
541 10.1002/(sici)1097-0134(19991101)37:2<204::aid-prot6>3.0.co;2-o.
542 37. Haining RL, Jones JP, Henne KR, Fisher MB, Koop DR, Trager WR, et al. Enzymatic
543 determinants of the substrate specificity of CYP2C9: role of B '− C loop residues in
544 providing the π-stacking anchor site for warfarin binding. Biochemistry 1999;38: 3285-
545 92. doi: 10.1021/bi982161+.
546 38. Melet A, Assrir N, Jean P, Lopez-Garcia MP, Marques-Soares C, Jaouen M, et al.
547 Substrate selectivity of human cytochrome P450 2C9: importance of residues 476, 365,
548 and 114 in recognition of diclofenac and sulfaphenazole and in mechanism-based
549 inactivation by tienilic acid. Arch Biochem Biophys 2003;409: 80-91. doi:
550 10.1016/s0003-9861(02)00548-9

551 39. Wester MR, Yano JK, Schoch GA, Yang C, Griffin KJ, Stout CD, et al. The structure
552 of human cytochrome P450 2C9 complexed with flurbiprofen at 2.0-A resolution. J
553 Biol Chem. 2004;279: 35630-7. doi: 10.1074/jbc.M405427200.
554 40. Afzelius L, Zamora I, Ridderström M, Andersson TB, Karlen A, Masimirembwa CM.
555 Competitive CYP2C9 inhibitors: enzyme inhibition studies, protein homology
556 modeling, and three-dimensional quantitative structure−actvity relationship analysis.
557 Mol Pharmacol. 2001;59: 909−19. doi: 10.1124/mol.59.4.909
558 41. Tanaka T, Kamiguchi N, Okuda T, Yamamoto Y. Characterization of the CYP2C8
559 active site by homology modeling. Chem Pharm Bull. 2004;52: 836-41. doi:
560 10.1248/cpb.52.836.
561 42. Gradishar WJ, Kaklamani V, Sahoo TP, Lokanatha D, Raina V, Bondarde S, et al. A
562 double-blind, randomised, placebo-controlled, phase 2b study evaluating sorafenib in
563 combination with paclitaxel as a first-line therapy in patients with HER2-negative
564 advanced breast cancer. Eur J Cancer. 2013;49: 312-22. doi:
565 10.1016/j.ejca.2012.08.005.
566 43. Miyata Y, Asai A, Mitsunari K, Matsuo T, Ohba K, Sakai H. Safety and efficacy of
567 combination therapy with low-dose gemcitabine, paclitaxel, and sorafenib in patients
568 with cisplatin-resistant urothelial cancer. Med Oncol. 2015;32: 235. doi:
569 10.1007/s12032-015-0683-y.
570 44. I Okamoto, M Miyazaki, R Morinaga, H Kaneda, S Ueda, Y Hasegawa, et al. Phase I
571 clinical and pharmacokinetic study of sorafenib in combination with carboplatin and
572 paclitaxel in patients with advanced non-small cell lung cancer. Invest New Drugs.
573 2010;28: 844-53. doi: 10.1007/s10637-009-9321-x.
574 45. Toubert ME, Vercellino L, Faugeron I, Lussato D, Hindie E, Bousquet G. Fatal heart
575 failure after a 26-month combination of tyrosine kinase inhibitors in a papillary thyroid
576 cancer. Thyroid. 2011;21: 451-4. doi: 10.1089/thy.2010.0270.
577 46. Nabhan C, Villines D, Valdez TV, Tolzien K, Lestingi TM, Bitran JD, et al. Phase I
578 study investigating the safety and feasibility of combining imatinib mesylate (Gleevec)
579 with sorafenib in patients with refractory castration-resistant prostate cancer. Br J
580 Cancer. 2012;107: 592-7. doi: 10.1038/bjc.2012.312.
581 47. Butler AM, Murray M. Inhibition and inactivation of constitutive cytochromes P450 in
582 rat liver by parathion. Mol Pharmacol. 1993;43:902-8.

583 48. Ortiz de Montellano PR, Correia MA. Suicidal destruction of cytochrome P-450 during
584 oxidative drug metabolism. Annu Rev Pharmacol Toxicol. 1983;23:481-503. doi:
585 10.1146/annurev.pa.23.040183.002405.
586 49. He K, Iyer KR, Hayes RN, Sinz MW, Woolf T, Hollenberg PF. Inactivation of
587 cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. Chem Res
588 Toxicol 1998;11:252-9. doi: 10.1021/tx970192k.
589 50. Murray M. Complexation of cytochrome P-450 isoenzymes in hepatic microsomes
590 from SKF 525-A-induced rats. Arch Biochem Biophys. 1988;262:381-8.
591 doi.org/10.1016/0003-9861(88)90202-0.
592 51. Murray M, Field SL. Inhibition and metabolite complexation of rat hepatic microsomal
593 cytochrome P450 by tricyclic antidepressants. Biochem Pharmacol. 1992;43:2065-71.
594 doi.org/10.1016/0006-2952(92)90163-D.
595 52. Murray M, Wilkinson CF, Marcus C, Dube CE. Structure-activity relationships in the
596 interactions of alkoxymethylenedioxybenzene derivatives with rat hepatic microsomal
597 mixed-function oxidases in vivo. Mol Pharmacol. 1983;24: 129-36.
598 53. Murray M, Butler AM, Stupans I. Competitive inhibition of human liver microsomal
599 cytochrome P450 3A-dependent steroid 6β-hydroxylation activity by
600 cyclophosphamide and ifosfamide in vitro. J Pharmacol Exp Ther. 1994;270: 645-9.
601 54. Murray M. In vitro effects of quinoline derivatives on cytochrome P-450 and
602 aminopyrine N-demethylase activity in rat hepatic microsomes. Biochem Pharmacol.
603 1984;33:3277-81. doi.org/10.1016/0006-2952(84)90090-X.
604 55. Ohyama K, Nakajima M, Suzuki M, Shimada N, Yamazaki H, Yokoi T. Inhibitory
605 effects of amiodarone and its N-deethylated metabolite on human cytochrome P450
606 activities: prediction of in vivo drug interactions. Br J Clin Pharmacol. 2000;49:244-53.
607 doi.org/10.1046/j.1365-2125.2000.00134.x.
608 56. Giri P, Naidu S, Patel N, Patel H, Srinivas NR. Evaluation of in vitro cytochrome P450
609 inhibition and in vitro fate of structurally diverse N-oxide metabolites: Case studies
610 with clozapine, levofloxacin, roflumilast, voriconazole and zopiclone. Eur J Drug
611 Metab Pharmacokinet. 2017;42:677-88. doi: 10.1007/s13318-016-0385-7.
612 57. Miller AA, Murry DJ, Owzar K, Hollis DR, Kennedy EB, Abou-Alfa G, et al. Phase I
613 and pharmacokinetic study of sorafenib in patients with hepatic or renal dysfunction:
614 CALGB 60301. J Clin Oncol. 2009;27:1800-5. doi: 10.1200/JCO.2008.20.0931.

58. Flaherty KT, Lathia C, Frye RF, Schuchter L, Redlinger M, Rosen M, et al. Interaction of sorafenib and cytochrome P450 isoenzymes in patients with advanced melanoma: a phase I/II pharmacokinetic interaction study. Cancer Chemother Pharmacol. 2011;68:1111-8. doi: 10.1007/s00280-011-1585-0.

59. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138:103-41. DOI: 10.1016/j.pharmthera.2012.12.007.

60. Murray M, Gillani TB, Ghassabian S, Edwards RJ, Rawling T. Differential effects of hepatic cirrhosis on the intrinsic clearances of sorafenib and imatinib by CYPs in human liver. Eur J Pharm Sci 2018;114:55-63. Doi: 10.1016/j.ejps.2017.12.003.

61. Cantrill E, Murray M, Mehta I, Farrell GC. Down-regulation of the male-specific steroid 16α-hydroxylase, cytochrome P-450$_{UT-A}$, in male rats with portal bypass: Relevance to estradiol accumulation and impaired drug metabolism in hepatic cirrhosis. J Clin Invest. 1989;83:1211-6. doi: 10.1172/JCI114003

.

630
631 **FIGURE LEGENDS**

632 Figure 1: SOR and its biotransformation to the principal metabolite SNO.

633 Figure 2: Inhibition of (a) paclitaxel 6α-hydroxylation and (b) losartan oxidation in human
634 hepatic microsomes by SOR (closed bars) and SNO (open bars). Different from
635 inhibition by SOR at that concentration: *P<0.05, ***P<0.001.

636 Figure 3: Kinetic analysis of the inhibition of human microsomal paclitaxel 6α-
637 hydroxylation by SOR and SNO. (a) Michaelis-Menten plot of paclitaxel 6α-
638 hydroxylation; (b) Lineweaver-Burk plot at SOR concentrations (●) 0 μM, (▲) 1
639 μM, (■) 10 μM, (O) 30 μM, (Δ) 50 μM, (c) Lineweaver-Burk plot at SNO
640 concentrations (●) 0 μM, (▲) 1 μM, (■) 10 μM, (O) 30 μM, (Δ) 50 μM, (d)
641 Lineweaver-Burk slope replots for SOR (■) and SNO (●). A representative
642 analysis conducted in microsomal fractions from donor liver HL9 is shown. Values
643 are means of at least duplicate determinations that varied by <12%.

644 Figure 4. Michaelis-Menten equilibria showing formation of CYP2C8-inhibitor/substrate
645 complexes and linear mixed-type reversible inhibition of CYP2C8-dependent
646 paclitaxel 6α-hydroxylation; [inhibitor] refers to either SOR or SNO.

647 Figure 5: Binding modes of (a) SOR and (b) SNO in X-ray structures of CYP2C8. Key
648 binding site residues are displayed (C atoms in green). C atoms in SOR and SNO
649 are represented as sticks in magenta and orange, respectively. The CYP heme is
650 shown in ball and stick format. O, N, F, Cl are shown in red, blue, cyan and green,
651 respectively.

652 Figure 6: Binding modes of (a) SOR and (b) SNO in X-ray structures of CYP2C9. Key
653 binding site residues are displayed (C atoms in green). C atoms in SOR and SNO
654 are represented as sticks in magenta and orange, respectively. The CYP heme is
655 shown in ball and stick format. O, N, F, Cl are shown in red, blue, cyan and green,
656 respectively.

657