Ticlopidine as a Selective Mechanism-Based Inhibitor of Human Cytochrome P450 2C19†

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ABSTRACT: Experiments using recombinant yeast-expressed human liver cytochromes P450 confirmed previous literature data indicating that ticlopidine is an inhibitor of CYP 2C19. The present studies demonstrated that ticlopidine is selective for CYP 2C19 within the CYP 2C subfamily. UV−visible studies on the interaction of a series of ticlopidine derivatives with CYP 2C19 showed that ticlopidine binds to the CYP 2C19 active site with a K_s value of 2.8 ± 1 μM. Derivatives that do not involve either the *o*-chlorophenyl substituent, the free tertiary amine function, or the thiophene ring of ticlopidine did not lead to such spectral interactions and failed to inhibit CYP 2C19. Ticlopidine is oxidized by CYP 2C19 with formation of two major metabolites, the keto tautomer of 2-hydroxyticlopidine (**1**) and the dimers of ticlopidine *S*-oxide (TSOD) (V_{max} = 13 ± 2 and 0.4 ± 0.1 min^{-1}). During this oxidation, CYP 2C19 was inactivated; the rate of its inactivation was time and ticlopidine concentration dependent. This process meets the chemical and kinetic criteria generally accepted for mechanism-based enzyme inactivation. It occurs in parralel with CYP 2C19-catalyzed oxidation of ticlopidine, is inhibited by an alternative well-known substrate of CYP 2C19, omeprazole, and correlates with the covalent binding of ticlopidine metabolite(s) to proteins. Moreover, CYP 2C19 inactivation is not inhibited by the presence of 5 mM glutathione, suggesting that it is due to an alkylation occurring inside the CYP 2C19 active site. The effects of ticlopidine on CYP 2C19 are very analogous with those previously described for the inactivation of CYP 2C9 by tienilic acid. This suggests that a similar electrophilic intermediate, possibly a thiophene *S*-oxide, is involved in the inactivation of CYP 2C19 and CYP 2C9 by ticlopidine and tienilic acid, respectively. The kinetic parameters calculated for ticlopidine-dependent inactivation of CYP 2C19, i.e., $t_{1/2max}$ = 3.4 min, k_{inact} = 3.2 10^{-3} s^{-1}, K_I = 87 μM, k_{inact}/K_I = 37 $L \cdot mol^{-1} \cdot s^{-1}$, and *r* (partition ratio) = 26 (in relation with formation of **1** + TSOD), classify ticlopidine as an efficient mechanism-based inhibitor although somewhat less efficient than tienilic acid for CYP 2C9. Importantly, ticlopidine is the first selective mechanism-based inhibitor of human liver CYP 2C19 and should be a new interesting tool for studying the topology of the active site of CYP 2C19.

The cytochromes P450 (CYP or P450)[1] play a key role in the metabolism of exogenous compounds such as drugs (*1*). To interpret or to predict various problems that may occur with some drugs in relation to genetic polymorphism and drug−drug interaction, it is very important to determine which human liver cytochrome P450 is involved in the metabolism of a given drug. This requires simultaneous approaches using hepatocytes, recombinant enzymes, and human liver microsomes in the presence of specific inhibitors of the various human P450s. Cytochromes P450 of the 3A and 2C subfamilies are the major isoforms present in human liver (*1*−*3*). Genetic analysis of the CYP 2C subfamily in humans has shown the presence of four major genes, *CYP 2C8*, *2C9*, *2C18*, and *2C19*, with several allelic variants. CYP 2C8, 2C9, and 2C19 are the 2C isozymes that are expressed at the highest level in human liver (*2*−*5*). CYP 2C9 and CYP 2C19 are involved in the metabolism of many drugs such as (*S*)-warfarin (*6*), diclofenac (*7*), and many nonsteroidal antiinflammatory agents (*8*−*13*) for the former and (*S*)-mephenytoin (*14*) and omeprazole (*15*, *16*) for the latter.

Specific, high-affinity inhibitors have been described for CYP 2C9. This is the case of sulfaphenazole, a competitive inhibitor for CYP 2C9 that exhibits a K_I value of 0.3 μM (*17*), and of tienilic acid (TA) (*18*), which acts as a selective

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[1] Abbreviations: CYP or P450, cytochrome P-450; TSOD, ticlopidine *S*-oxide dimer; DLPC, dilauroyl-L-α-phosphatidylcholine; TA, tienilic acid; TPP, 3-[2,3-dichloro-4-(2-thenoyl)phenoxy]propan-1-ol; TPE, 2-[2,3-dichloro-4-(2-thenoyl)phenoxy]ethanol; GSH, reduced glutathione; SOD, superoxide dismutase.

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suicide substrate of CYP 2C9 (*19*). Many fewer tools are available for CYP 2C19. However, recent reports have indicated that ticlopidine, a drug that exhibits a wide spectrum of platelet antiaggregating activity in man (*20*), is able to inhibit CYP 2C19. Thus, recent case reports have shown that ticlopidine inhibits phenytoin clearance (*21*−*24*). Phenytoin is metabolized by CYP 2C9 and 2C19 (*25*), and ticlopidine decreases the activity of CYP 2C19 in vivo whereas it does not affect CYP 2C9 activity (*26*, *27*). Moreover, in vitro inhibitory effects of ticlopidine on bufuralol (*28*) and (*S*)-mephenytoin (*29*) hydroxylation catalyzed by recombinant CYP 2C19 have been described.

Studies have been performed in our laboratory in order to know whether ticlopidine is a selective inhibitor of CYP 2C19 within the CYP 2C subfamily in human liver and to determine the mechanism of its inhibition and the structural factors that are important for its effects toward CYP 2C19. This paper shows that ticlopidine is a selective mechanism-based inhibitor of CYP 2C19. Several mechanism-based inhibitors of cytochromes P450 from the 1A, 2A, 2B, 2E, and 3A subfamilies have been reported (*30*−*36*); however, none has been described so far for CYP 2C19. Ticlopidine behaves toward CYP 2C19 in a manner similar to that of tienilic acid toward CYP 2C9 and appears as a useful tool for studying the topology of the human CYP 2C19 active site.

MATERIALS AND METHODS

Chemicals. All chemicals used were of the highest quality commercially available. Dilauroyl-L-α-phosphatidycholine (DLPC), dextromethorphan, testosterone, 7-ethoxyresorufin, ticlopidine, superoxide dismutase (SOD), and catalase were purchased from Sigma. TA was provided by Anphar-Rolland (Chilly-Mazarin, France). 3-[2,3-dichloro-4-(2-thenoyl)phenoxy]propan-1-ol (TPP) and 2-[2,3-dichloro-4-(2-thenoyl)-phenoxy]ethanol (TPE) were prepared by previously described procedures (*37* and *12*, respectively). Compounds **1**, **2**, and **3** were obtained from Sanofi (Toulouse, France). [4-^{14}C]Ticlopidine (specific activity 33.6 mCi/mmol) was obtained from Sanofi (Toulouse, France). Its radiochemical purity was checked by HPLC and found to be higher than 98%.

Physical Measurements. UV−visible spectra were recorded on a Kontron Uvikon 860 spectrophotometer equipped with a diffusion sphere. ^{1}H NMR spectra were recorded at 27 °C on a Bruker ARX-250 instrument; chemical shifts are reported downfield from $(CH_3)_4Si$ and coupling constants are in hertz. The abbreviations s, d, t, m, bs, and dd are used for singlet, doublet, triplet, multiplet, broad singlet, and doublet of doublets, respectively. Mass spectra (MS) were performed with chemical ionization (CI) using NH_3 on a Nermag R1010 apparatus. Compound **4** contains two chlorine atoms, and all peaks corresponding to molecular ions or fragments involving two Cl atoms exhibited the isotope cluster expected for the presence of two Cl atoms (with a M:M + 2:M + 4 ratio of 10:6:1); *m*/*z* values described below are for ^{35}Cl. Elemental analyses were carried out at Centre Regional de Microanalyse, Paris.

Synthesis of Ticlopidine Derivatives (Figure 1). (A) Bis-(2-chlorobenzyl)methylamine (**4**). 2-Chlorobenzyl chloride (15 mL, 0.118 mol) in 5 mL of absolute ethanol was added dropwise to an ice-cold solution of methylamine (0.12 mol) in 40 mL of absolute ethanol. The mixture was stirred for 20 h at room temperature. After evaporation of alcohol, the residue was dissolved in 1 N HCl; unreacted chlorobenzyl chloride was extracted with CH_2Cl_2. Concentrated NaOH was then added, and amines were extracted with CH_2Cl_2, dried over Na_2SO_4, and separated by column chromatography (SiO_2, CH_2Cl_2, and then a stepwise gradient elution from 5% to 12% methanol). Bis(2-chlorobenzyl)methylamine (1.8 g) was obtained as an oil (10.8% yield). ^{1}H NMR ($CDCl_3$): 7.53 (dd, 2H, J = 7.4, J = l.9), 7.32 (dd, 2H, J = 7.4, J = 1.6), 7.22 (dt, 2H, J = 7.4, J = 1.9), 7.15 (dt, 2H, J = 7.4, J = 1.6), 3.70 (s, 4H, $2CH_2$), 2.24 (s, 3H, CH_3). MS (CI, NH_3): m/z = 280 ($[M + H]^+$, 100%).

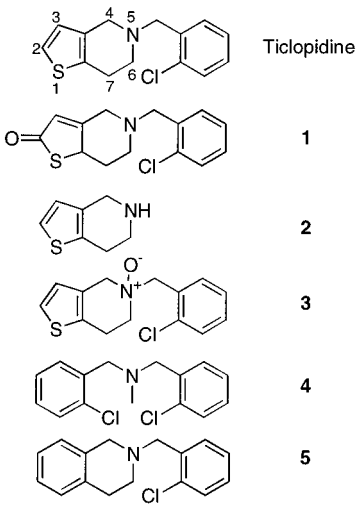


FIGURE 1: Structure of the ticlopidine derivatives used in this study.

(B) 2-(2-Chlorobenzyl)-1,2,3,4-tetrahydroisoquinoline (**5**). To a solution of 1,2,3,4-tetrahydroisoquinoline (2.5 mL, 0.02 mmol) in absolute ethanol (10 mL) was added dropwise 2-chlorobenzyl chloride (2.6 mL, 0.02 mmol) in 5 mL of absolute ethanol. The mixture was stirred overnight at room temperature and for 4 h at 70 °C. Alcohol was evaporated, and the residue dissolved in water was adjusted to pH 10 with 1 N NaOH, extracted with CH_2Cl_2, dried over $MgSO_4$, and purified by column chromatography (SiO_2, CH_2Cl_2, and then 10% $EtOCOCH_3$). 2-(2-Chlorobenzyl)-l,2,3,4-tetrahydroisoquinoline (5 g) was obtained as an oil (97% yield). ^{1}H NMR ($CDCl_3$): 7.58 (d, 1H), 7.38 (d, 1H), 7.28−7.18 (m, 2H), 7.13 (bs, 3H), 7.02 (bs, 1H), 3.81 (s, CH_2), 3.72 (s, CH_2), 2.93 (t, CH_2), 2.82 (t, CH_2). Anal. Calcd for $C_{16}H_{16}ClN$: C, 74.56; H, 6.26; N, 5.43. Found: C, 74.40; H, 6.30; N, 5.33.

Origins of Recombinant Human Liver Cytochromes P450. The first system was used for expression not only of CYP 2C19 but also of human liver CYP 1A2, 2C8, 2C9, 2C18, 2D6, and 3A4. It is based on previously described yeast strain W(R)fur1 (*38*), in which yeast cytochrome P450 reductase was overexpressed. Transformation by a pYeDP60 vector containing one of the human liver CYP 1A2, 2C8, 2C9, 2C18, 2C19, 2D6, and 3A4 cDNAs (*39*−*42*) was then performed according to a general method of construction of yeast strain W(R)fur 1 expressing various human liver P450s (*43*, *44*). Yeast culture and microsome preparation were performed by using previously described techniques (*45*). Microsomes were homogenized in 50 mM Tris buffer (pH

= 7.4) containing 1 mM EDTA and 20% glycerol (v/v), aliquoted, frozen under liquid N_2, and stored at −80 °C until use. P450 contents of yeast microsomes were 100, 40, 90, 40, 20, 40, and 200 pmol of P450/mg of protein for CYP 1A2, 2C8, 2C9, 2C18, 2C19, 2D6, and 3A4, respectively.

Another system was used in the particular case of CYP 2C19. It consists of microsomes prepared from insect cells that were infected with a baculovirus containing the cDNA coding for CYP 2C19 and rabbit cytochrome P450 reductase. These microsomes were purchased from Panvera (Madison, WI). P450 2C19 and P450 reductase contents of these microsomes were 113 and 640 pmol/mg of protein, respectively. Purified CYP 2C19 (*48*) and rat liver cytochrome P450 reductase (*7, 12*) were prepared as described previously. Microsomal P450 content was determined according to the method of Omura and Sato (*46*). The protein content in microsomal suspensions was determined by the Lowry procedure (*47*) using bovine serum albumin as the standard.

Enzyme Activity Assay. (A) Ethoxyresorufin O-Deethylation. The 7-ethoxyresorufin *O*-deethylase activity of yeast microsomes was measured at 28 °C using a previously described method (*49*).

(B) 6β-Hydroxylation of Testosterone. The assay for testosterone 6β-hydroxylation (*50*) was carried out using cytochrome b_5 (0.4 μM cytochrome b_5 for 0.2 μM P450). The reaction proceeded for 10 min at 28 °C.

(C) Dextromethorphan Demethylation. The assay for dextromethorphan demethylation (*51*) was carried out using 8.8 μM dextromethorphan. The reaction proceeded for 10 min at 28 °C.

(D) 5-Hydroxylation of 2-Aroylthiophenes. Quantitation of 5-hydroxy-2-aroylthiophenes was based on a spectrophotometric method (*52*) adapted to yeast microsomes expressing CYP 2C9 in the case of TA (*18*). Incubations for metabolic activity with yeast microsomes were carried out at 28 °C, using glass tubes in a shaking bath. The incubation mixtures contained the yeast microsomal suspension, providing 0.2, 0.2, 0.1, and 0.075 μM P450 for CYP 2C8, 2C9, 2C18, and 2C19 respectively, the substrate (500 μM TPE, 12 μM TA, 30 μM TPP, and 200 μM TPP for CYP 2C8, 2C9, 2C18, and 2C19 respectively), and a NADPH-generating system (1 mM $NADP^+$, 10 mM glucose 6-phosphate, and 2 units of glucose 6-phosphate dehydrogenase/mL) diluted in 0.1 M Tris buffer, pH = 7.4, containing 1 mM EDTA and 8% glycerol (final concentrations). Activity assays were routinely initiated (t_0 = 0 min) by addition of the NADPH-generating system into the incubation mixture after 3 min of separate preincubation at 28 °C for temperature equilibration. At t_0 and regularly after, aliquots (140 μL) were taken, and the reaction was quickly stopped by treatment with 70 μL of a cold CH_3CN/CH_3COOH (10:1) mixture.

Study of Substrate Binding to Purified CYP 2C19 by Difference Visible Spectroscopy. Binding spectra of ticlopidine and its analogues were recorded at room temperature with an Aminco DW2 spectrometer modified by Olis Instrument Inc. Dilauroyl-L-α-phosphatidylcholine (30 μg/200 pmol of P450) and 200 pmol of purified P450 were combined on ice and preincubated at 4 °C for 30 min. The mixture was diluted to a final concentration of 0.2 μM P450 in 0.1 M phosphate buffer, pH = 7.4. The solution was equally divided between two 500 μL black quartz cuvettes (1 cm path length), and a baseline was recorded. Aliquots (1−5 μL) of solutions containing the studied compound were added to the sample cuvette, the same volume of solvent being added to the reference cuvette. The difference spectra were recorded between 350 and 500 nm (*53*).

Study of CYP 2C19 Inactivation by Ticlopidine. (A) General Incubation Conditions. Incubations for metabolic activity with insect microsomes containing recombinant CYP 2C19 were carried out at 37 °C, using glass tubes in a shaking bath. The incubation mixtures contained the microsomal suspension, providing 0.1 μM P450, the substrate (as indicated in experiments), and a NADPH-generating system (1 mM $NADP^+$, 10 mM glucose 6-phosphate, and 2 units of glucose 6-phosphate dehydrogenase/mL) diluted in 0.1 M phosphate buffer, pH = 7.4, containing 0.1 mM EDTA. Activity assays were routinely initiated (t_0 = 0 min) by incorporation of the NADPH-generating system into the incubation mixture after 3 min of separate preincubation at 37 °C for temperature equilibration. These conditions apply, unless noted otherwise, to all of the experiments described below.

(B) HPLC Analysis of the Oxidation of Ticlopidine by Human CYP 2C19-Expressed Insect Microsomes. Ticlopidine was incubated at 37 °C in the presence of insect microsomes (0.1 μM CYP 2C19) in 0.1 M phosphate buffer, pH = 7.4, containing 0.1 mM EDTA and a NADPH-generating system. Activity assays were routinely initiated (t_0 = 0 min) by incorporation of the NADPH-generating system into the incubation mixture after 3 min of separate preincubation at 37 °C for temperature equilibration. At t_0 and regularly after, aliquots (140 μL) were taken, and the reaction was quickly stopped by treatment with 70 μL of a cold CH_3CN/CH_3COOH (10:1) mixture. Proteins were precipitated by centrifugation for 5 min at 10 000 rpm, and the supernatant was stored at −40 °C until analysis. Formation of the metabolites was followed by reverse-phase HPLC (Spectra system AS 3000 autosampler). Supernatant aliquots were injected onto a Kromasil C18 column (ColoChrom, Gagny, France) (150 × 4.6 mm, 5 μm). The mobile phase was delivered at a rate of 1 mL/min with a Spectra Physics system P4000 pump with a gradient from A (0.1 M acetate, pH 4.6) to B ($CH_3CN/CH_3OH/H_2O$, 7:2:1): A/B 0% up to 100% in 20 min. Monitoring of the column effluent was performed with a scanning Spectra Focus UV detector between 220 and 340 nm and measurement of product concentrations at 240 nm. In that system, retention times for ticlopidine and its metabolites **1** and TSOD were 22, 19, and 21 min, respectively.

(C) Incubations for Inactivation Kinetics. The experimental design to determine the rate of enzyme inactivation was based on the general procedures previously described for other P450 suicide substrates in liver microsomes (*19, 54, 55*). Basically, microsomes (equivalent to 0.3 μM P450) were incubated in the conditions outlined above with various concentrations of ticlopidine (ranging from 1 to 100 μM). At t_0 and regularly after (from 0 to 30 min), parallel aliquots were removed from the incubation medium and were immediately processed to determine ticlopidine metabolite formation (140 μL) and residual enzymatic activity (30 μL) as described later. The same procedure was also used in experiments aimed at correlating the time and ticlopidine concentration dependence of ticlopidine metabolite formation with enzyme inactivation. In all instances, control incubations

were run in parallel with the experimental variables to correct for the possible contribution of microsomal ability to time-dependent loss of activity. For this purpose, microsomes were incubated in the general conditions described but under noncatalysis conditions, i.e., lacking either the substrate (100 μM ticlopidine) (with or without 100 units/mL SOD and 100 units/mL catalase) or the NADPH-generating system. Aliquots were taken regularly (from t_0) for the immediate determination of the remaining monooxygenase activity (as described later).

(D) Measurement of the Remaining Enzymatic Activity. Routine experimental procedures to determine the enzymatic activity remaining upon exposure to a suicide substrate (*54*, *55*) incorporate the use of an alternative substrate to assay the enzymatic activity in a second incubation period. This requires the prior elimination of the inactivator substrate already present in the aliquots, which otherwise could interfere in the accurate determination of enzyme residual activity. This is routinely achieved by a 10−100-fold dilution of the sample in the standard assay medium, which minimizes the interference of the inactivator with the second substrate. Briefly, 30 μL aliquots from the experiments of CYP 2C19 inactivation were rapidly diluted in a total volume of 300 μL, containing TPP and the NADPH-generating system. An aliquot (140 μL) was taken and immediately quenched by treatment with 70 μL of the cold CH_3CN/CH_3-COOH (10:1) mixture. The remaining medium was incubated, and another aliquot (140 μL) was removed at $t = 10$ min and treated as the first one. In the case of inactivation experiments using yeast microsomes expressing CYP 2C8 and 2C9, a similar method was used with TPE and TA as substrates for CYP 2C8 and 2C9, respectively, in 0.1 M Tris buffer, pH = 7.4, containing 1 mM EDTA and 8% glycerol. For CYP 2C8 and 2C9, 300 μL samples of the primary reaction mixture were removed and mixed with 3 μL of a concentrated substrate solution and incubated at 28 °C for 10 or 5 min for CYP 2C8 and 2C9, respectively. Enzyme activity was stopped as described above.

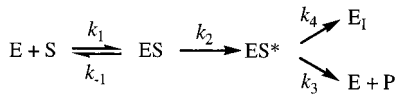
The rate of 5-hydroxylation of 2-aroylthiophenes was determined as previously reported (*18*); 5-OHTPP, 5-OHTPE, or 5-OHTA formation was determined by recording the difference spectrum ($A_{380} - A_{520}$) of the sample against a baseline performed with the t_0 sample [$\epsilon_{390} = 28000\ M^{-1}\ cm^{-1}$ (*52*)] using 100 μL black-masking quartz microcuvettes (Hellma, Mulheim, Germany). This procedure applies for all of the determinations noted as "remaining activity" in the referenced experiments. In all instances, the initial 5-hydroxylation rate was taken as a measure of the maximal enzyme activity (100% activity).

(E) Protective Effect of Omeprazole. Incubations were carried out as decribed in section C in order to determine the time course of enzyme inactivation in the presence of omeprazole. Omeprazole (100 μM) was added (or not) at t_0 of exposure to 50 μM ticlopidine. At the indicated times, the residual activity was determined as described above. No significant differences were found in the maximal hydroxylation rate (100% at t_0 incubation), whether omeprazole was present in the assay for residual activity [1.3 ± 0.2 nmol of 5-OHTPP (nmol of P450)$^{-1}$ min^{-1} vs 1.2 ± 0.2 nmol of 5-OHTPP (nmol of P450)$^{-1}$ min^{-1} with or without omeprazole respectively]. Controls under noncatalysis conditions were also run in parallel with the experimental incubations.

(F) Effect of GSH on the Rate of Inactivation. The time dependence of CYP 2C19 inactivation upon oxidation of 50 μM ticlopidine was measured according to the general procedure described, with or without 5 mM GSH in the incubation mixture. The enzyme activity remaining was monitored in the standard way by aliquots taken regularly (from 0 to 30 min) from the incubation mixture. Control incubations containing the NADPH-generating system with GSH and without ticlopidine added were also run in parallel.

(G) Covalent Binding Measurement and Stoichiometry of the Inactivation. Dilauroyl-L-α-phosphatidycholine (30 μg/mL), 0.5 μM purified CYP 2C19, and 0.5 μM rat liver cytochrome P450 reductase were combined on ice and preincubated at 37 °C for 5 min. The incubation mixtures contained this reconstitued system, 100 μM [^{14}C]ticlopidine, 5 mM GSH, and a NADPH-generating system (1 mM $NADP^+$, 10 mM glucose 6-phosphate, and 2 units of glucose 6-phosphate deshydrogenase/mL) diluted in 0.1 M phosphate buffer, pH = 7.4, containing 0.1 mM EDTA. Inactivation was allowed to proceed until the residual activity was minimal (about 30 min). During this period, aliquots were taken regularly in which covalent binding of ticlopidine metabolites to protein (50 μL) and the remaining activity (30 μL) were determined in parallel. The remaining activity was measured by the described procedures. At the indicated times, a 50 μL aliquot was spotted on a glass fiber filter (GFB Whatman) for covalent binding measurements. These measurements were done according to previously described procedures (*56*).

(H) Kinetic Analyses. Kinetic parameters of the inactivation process were calculated according to the models of Walsh (*57*) and Silvermann (*54*). This process can be represented in the simplest way by the equation:



where ES is the initial enzyme−substrate complex converted to an activated species, ES*. S* (the electrophilic ticlopidine intermediate) can either react within the complex to produce E_I (the irreversibly inhibited enzyme) or break down to yield free enzyme (E) and products (P).

k_{-1}/k_1 represents the K_s (dissociation constant), and k_3/k_4 is the partition ratio between the number of productive turnovers and those leading to enzyme inactivation. The pseudo-first-order rate constant for the inactivation process (k_{inact}) is $k_2k_4/(k_2 + k_3 + k_4)$. The K_I constant, a term used for mechanism-based enzyme inactivators, is $[(k_{-1} + k_2)/k_1][(k_3 + k_4)/(k_2 + k_3 + k_4)]$. Curve fitting was performed by a single regression fit procedure of Cricket Graph (Cricket Software, Malvern, PA) on a Macintosh personal computer.

RESULTS

Inhibitory Effects of Ticlopidine on Recombinant Human Liver Cytochromes P450. The effects of ticlopidine on typical activities catalyzed by microsomes of yeast expressing human liver CYP 1A2, 2C8, 2C9, 2C18, 2C19, 2D6, and 3A4 were compared by using substrate concentrations equal to twice the K_M value for each enzyme. Table 1 shows that ticlopidine acts as a particularly good inhibitor of CYP 2C19 and 2D6 ($IC_{50} \sim 10\ \mu M$), in agreement with previously reported data

Table 1: Inhibitory Effects of Ticlopidine on Typical Oxidations Catalyzed by Yeast-Expressed, Recombinant Human Liver Cytochromes P450[a]

	P450						
	1A2	2C8	2C9	2C18	2C19	2D6	3A4
IC_{50} (μM)	75 ± 15	100 ± 20	>200[b]	100 ± 15	10 ± 5	10 ± 3	50 ± 10

[a] Oxidations of typical substrates of each enzyme were carried out at substrate concentrations equal to 2 K_M (conditions in Materials and Methods). Microsomes of W(R)furl yeast expressing each enzyme were used under conditions described in Materials and Methods. [b] No inhibitory effect even at 200 μM ticlopidine. Values are mean ± SD from three to five experiments.

Table 2: Effects of Various Analogues of Ticlopidine on the Visible Spectrum of CYP 2C19 and on the 5-Hydroxylation of TPP Catalyzed by This Cytochrome

compound	spectral effects[a]	K_s (μM)	IC_{50} (μM)[b]
ticlopidine	type I	2.8 ± 1	10 ± 5
1	type I	3.4 ± 1	20 ± 5
2	nd		>250
3	nd		>250
4	nd		>250
5	nd		>250

[a] Type of difference visible spectrum observed upon addition **of** compounds to purified, recombinant CYP 2C19 (0.2 μM); classical type I spectra correspond to the appearance of a peak at 390 nm and a trough at 420 nm; nd means that no significant difference spectra could be obtained even with 500 μM compound. [b] IC_{50} values determined as described in Materials and Methods using microsomes of yeast expressing CYP 2C19; >250 means activity higher than 50% control activity at 250 μM. Values are the mean ± SD from three to six experiments.

(*29*). On the contrary, it only exhibits weak effects on the other members of the human CYP 2C subfamily.

Study of the Interaction of Ticlopidine with CYP 2C19 by Visible Spectroscopy. Addition of ticlopidine to purified recombinant CYP 2C19 or to commercially available microsomes from insect cells expressing recombinant CYP 2C19 (Panvera, Madison, WI) led to the appearance of a difference visible spectrum characterized by a peak at 390 nm and a trough at 420 nm (data not shown). This typical type I difference spectrum (*53*) corresponds to the binding of ticlopidine in the CYP 2C19 active site, which leads to the removal of the H_2O sixth ligand of the iron and formation of a pentacoordinated CYP 2C19 iron(III). The dissociation constant calculated for the CYP 2C19−ticlopidine complex from these spectral studies was $K_s = 2.8 \pm 1$ μM (Table 2).

Comparison of the Inhibitory Effects and Spectral Interactions with CYP 2C19 of Ticlopidine Derivatives. Several derivatives of ticlopidine (Figure 1) have been compared in order to determine the structural factors that are important for recognition by CYP 2C19. Some of them, such as **1**, **2**, and **3**, were previously described as in vivo metabolites of ticlopidine (*58*). Compounds **4** and **5** have been synthesized in order to determine the importance of the thiophene ring for recognition by CYP 2C19. Their synthesis and structure determination are described in Materials and Methods. Table 2 shows that only two compounds, ticlopidine and its metabolite **1**, led to the appearance of a significant difference spectrum with recombinant, purified CYP 2C19. Interestingly, only these two compounds acted as good inhibitors of CYP 2C19 with IC_{50} values of 10 and 20 μM, respectively. All of the other compounds exhibited IC_{50} values larger than 250 μM. Lack of recognition of compound **5**, which only differs from ticlopidine by replacement of its thiophene ring by a benzene ring, shows that the thiophene ring of ticlopidine is crucial for efficient binding to CYP 2C19.

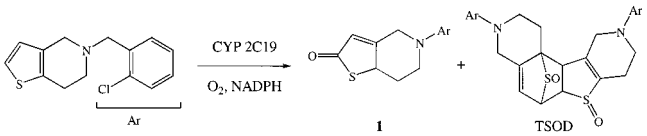


FIGURE 2: Structure of two metabolites formed upon CYP 2C19-catalyzed oxidation of ticlopidine. In fact, the dimer of ticlopidine *S*-oxide (TSOD) should exist as a mixture of stereoisomers. HPLC profiles of incubation mixtures coming from chemical or CYP 2C19-catalyzed oxidation of ticlopidine revealed the formation of a major stereoisomer, whose configuration was not yet established. Literature data on the formation of thiophene *S*-oxide dimers from oxidation of thiophene derivatives (*64*−*67*) suggest an endo-syn configuration of this stereoisomer of TSOD.

Oxidation of Ticlopidine by Recombinant CYP 2C19. Oxidation of ticlopidine with microsomes from insect cells expressing CYP 2C19 in the presence of a NADPH-generating system led to the formation of six metabolites, as shown by HPLC. The two major metabolites were identified (Figure 2). The first one exhibited an UV−visible spectrum and an HPLC retention time identical to those of an authentic sample of compound **1**, which should come from a hydroxylation at position 2 of the thiophene ring of ticlopidine. The second metabolite exhibited an UV−visible spectrum and an HPLC retention time identical to those of the ticlopidine *S*-oxide dimer (TSOD), which has been recently prepared by oxidation of ticlopidine with *m*-chloroperbenzoic acid in the presence of boron trifluoride etherate and completely identified by ^{1}H NMR and mass spectroscopy and elemental analysis (D. Amar, P. Dansette, and D. Mansuy, to be published elsewhere). The same metabolites were formed in incubations of ticlopidine with microsomes of yeast expressing CYP 2C19. However, their formation was not detected in incubations using microsomes of the W(R)fur1 yeast not expressing CYP 2C19 or in incubations not containing NADPH.

Time course formation of the two major metabolites of ticlopidine showed a short linear period (5 min) followed by a fast decrease of the reaction rate (Figure 3). Treatment of the kinetic data based on initial reaction rates led to K_M values of 46 ± 10 and 67 ± 10 μM and k_{cat} values of 13 ± 2 and 0.4 ± 0.1 min^{-1} for the formation of **1** and TSOD, respectively.

Effects of Ticlopidine Oxidation on CYP 2C19, 2C8, and 2C9. To further analyze the fast decrease of CYP 2C19 activity that was observed during ticlopidine oxidation (Figure 3), microsomes containing recombinant CYP 2C19 were preincubated with ticlopidine in the presence or absence of NADPH (i.e., under catalysis or noncatalysis conditions), and the remaining enzyme activity was measured as a function of the preincubation time.

Incubation of microsomes from insect cells expressing CYP 2C19 with 100 μM ticlopidine in the presence of NADPH led to a progressive loss of CYP 2C19 activity as a function of time (Figure 4A). Fifty percent of the activity was lost after 5 min, and only 10% remained after 30 min. Incubations under identical conditions but in the absence of

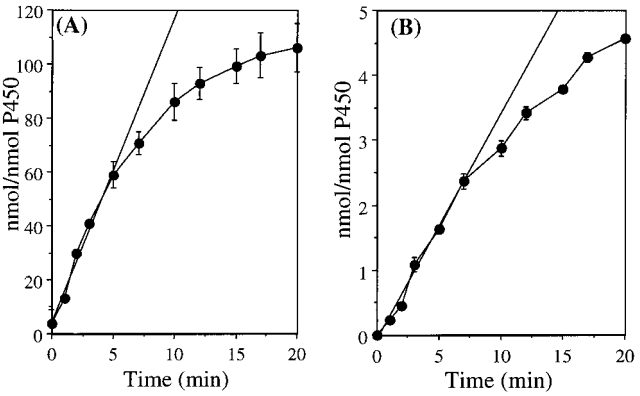


FIGURE 3: Time course formation of metabolite **1** (A) and TSOD (B) upon oxidation of ticlopidine by microsomes from insect cells expressing CYP 2C19. Microsomes from insect cells expressing CYP 2C19 were incubated under catalysis conditions in the presence of 100 μM ticlopidine. The amounts of metabolite **1** (A) and TSOD (B) formed were determined by HPLC analyses as detailed in Materials and Methods. Data correspond to the mean ± SD of three experiments.

NADPH did not lead to any loss of CYP 2C19 activity. In the absence of ticlopidine, only 25% of the activity was lost after 30 min incubation (Figure 4A). These results confirmed the existence of a catalysis-dependent inactivation of CYP 2C19 upon oxidation of ticlopidine. Interestingly, identical experiments performed with metabolite **1** instead of ticlopidine did not show any inactivation of CYP 2C19 (data not shown).

Identical experiments performed with microsomes from yeast expressing CYP 2C8 and 2C9, which are the two other members of the CYP 2C subfamily expressed at a detectable level in human liver (*2*, *59*, *60*), gave very different results. Incubation of microsomes from yeast expressing CYP 2C9 in the presence of NADPH, with or without ticlopidine, did not lead to any significant loss of activity of this isozyme (Figure 4B). Identical experiments performed on microsomes from yeast expressing CYP 2C8 led to a small, progressive decrease of CYP 2C8 activity, which was not dependent on the presence of ticlopidine (Figure 4C). It thus appeared that enzyme inactivation due to metabolic oxidation of ticlopidine was specific to CYP 2C19.

Kinetics of CYP 2C19 Inactivation by Ticlopidine. The general criteria required for proving that inactivation of an enzyme is due to a mechanism-based process (*54*, *57*) were then studied. Figure 5 shows that the loss of CYP 2C19 activity as a function of time for incubations in the presence of NADPH and various ticlopidine concentrations showed the classical biphasic kinetics described for other P450 suicide substrates (*19*, *30*−*36*, *55*). The time required for half-maximal inactivation, $t_{1/2}$, as well as the apparent first-order constant, k_{inact}, was calculated from the plot of the logarithmic transformation of the remaining activity as a function of time, as depicted in Figure 5B. Plots of $t_{1/2}$ versus reciprocal ticlopidine concentration (Figure 5, inset) led to the kinetic constants of the inactivation process. From extrapolation to infinite ticlopidine concentration, the time required to inactivate half of the enzyme at the maximal rate, $t_{1/2max}$, and the maximal k_{inact} were 3.4 ± 1 min and (3.2 ± 0.6) × 10^{-3} s^{-1}, respectively. The dissociation constant, K_I, was found to be 87 ± 30 μM, a value similar to the K_M values calculated for the CYP 2C19-dependent oxidation of ticlopidine to **1** and TSOD (46 ± 10 and 67 ± 10 μM; see above). The second-order rate constant, k_{inact}/K_I, a proposed index of the in vitro effectiveness of a substrate as inactivator (*54*, *57*), was found to be 37 ± 15 $L \cdot mol^{-1} \cdot s^{-1}$.

Figure 6 illustrates the correlation between the CYP 2C19 activity remaining after ticlopidine oxidation and the amount of metabolites, **1** and TSOD, formed for various ticlopidine concentrations and incubations times. A linear relationship was observed with the formation of both metabolites. This shows that the loss of CYP 2C19 activity parralleled the oxidative biotransformation of ticlopidine that results in the formation of **1** and TSOD. These data allowed us to estimate the partition ratio of the inactivation process, *r*, which represents the number of productive turnovers (leading to TSOD and **1**) divided by the number of inactivating events (*54*, *57*). Actually, extrapolation to 0% remaining activity in Figure 6 gave a *r* value of 4 ± 1 and 22 ± 5 in relation with the formation of TSOD and **1**, respectively. The *r* value in relation with TSOD formation takes into account that two turnovers are required for the formation of 1 mol of TSOD.

Protection of CYP 2C19 by Omeprazole. The presence of omeprazole, a well-known CYP 2C19 substrate (*15*, *16*), in incubations of CYP 2C19 with ticlopidine and NADPH led to a clear decrease of the rate of inactivation of CYP 2C19 (Figure 7).

Effects of Glutathione on CYP 2C19 Inactivation. An important property of efficient mechanism-based inhibitors is to generate reactive species that prefer to rapidly react within the active site than to diffuse out into solution. Figure 8 shows that the presence of 5 mM glutathione in the incubation medium did not have any significant effects on the rate of CYP 2C19 inactivation during oxidation of ticlopidine.

Relationship between Alkylation of CYP 2C19 and Its Inactivation. Incubation of [^{14}C]ticlopidine with a reconstituted system consisting in purified, recombinant CYP 2C19 and rat liver P450 reductase in the presence of a NADPH-generating system led to an important covalent binding of ticlopidine metabolites to proteins (5 ± 1 nmol/nmol of CYP 2C19 after 30 min, when using 100 μM ticlopidine, 0.5 μM CYP 2C19, and 0.5 μM reductase). This covalent binding clearly decreased in the presence of GSH in the incubation medium and reached a level of 1.7 nmol/nmol of CYP 2C19 with 5 mM GSH. Figure 9 shows that CYP 2C19 inactivation and covalent binding of ticlopidine metabolite(s) occurred simultaneously as a function of time. Approximately 1.7 nmol of ticlopidine metabolite(s) was covalently bound per nanomole of CYP 2C19 when 74% enzyme inactivation has occurred.

DISCUSSION

Our results using yeast-expressed human liver cytochromes P450 confirm previous literature data showing that ticlopidine is a good inhibitor of CYP 2C19 and CYP 2D6 (*28*, *29*). They show that this compound is a selective inhibitor of CYP 2C19 within the human liver CYP 2C subfamily (Table 1). Spectral interaction studies demonstrate that ticlopidine readily binds to the protein active site of CYP 2C19 (type I interaction) with a K_s of 2.8 μM (Table 2).

A comparison of the inhibitory effects and spectral interactions of five analogues of ticlopidine (Figure 1 and

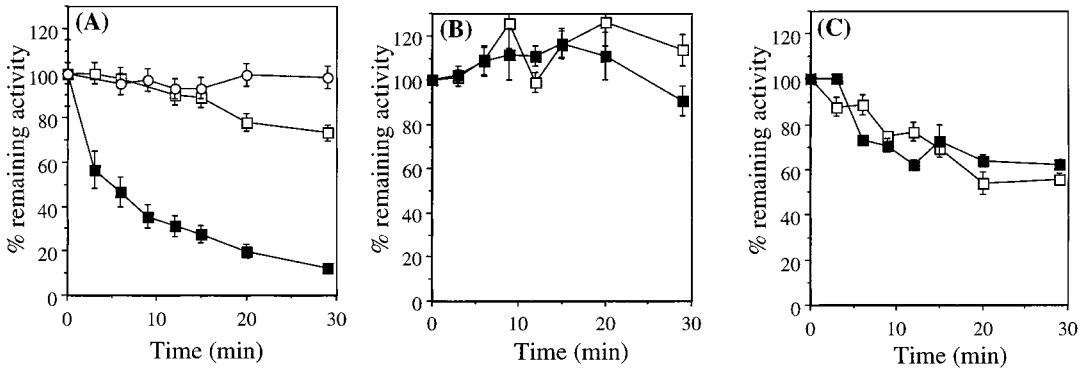


FIGURE 4: Time dependence of CYP 2C19 inactivation upon NADPH-dependent oxidation of ticlopidine (A) and comparison with CYP 2C9 (B) and 2C8 (C). Residual activities of each P450 were measured as described in Materials and Methods, after different times of incubation with microsomes and a NADPH-generating system, in the presence (■) or absence (□) of 100 μM ticlopidine. Control incubations (○) with CYP 2C19 (A) were performed with 100 μM ticlopidine without a NADPH-generating system.

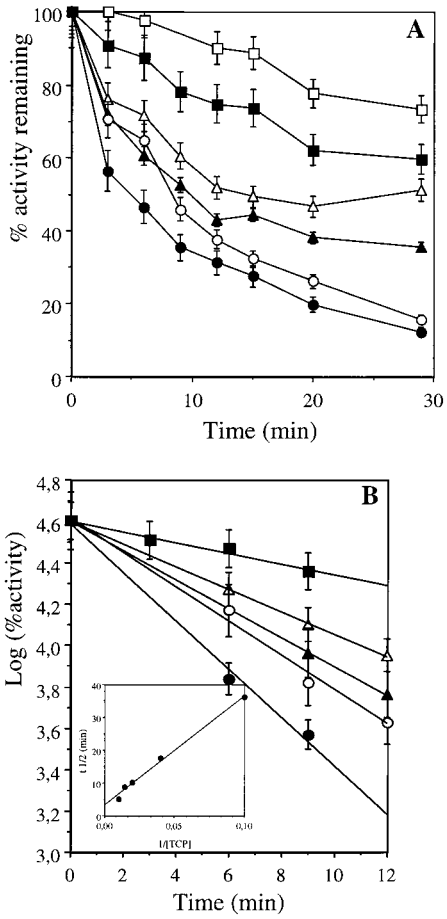


FIGURE 5: Kinetics of inactivation of CYP 2C19 by different concentrations of ticlopidine. Details for incubation and determination of the remaining activity have already been given in the Materials and Methods section. (A) Microsomes from insect cells expressing CYP 2C19 were incubated for the indicated periods in the presence of a NADPH-generating system and 10 (■), 25 (△), 50 (▲), 70 (○), or 100 μM (●) ticlopidine. Each point represents the mean and standard deviation from three separate experiments. (B) Linear regression analysis of the natural logarithm of the remaining CYP 2C19 activity as a function of time allowed the calculation of the time required for half-inactivation of the enzyme upon incubation in the presence of various ticlopidine concentrations [10 (■), 25 (△), 50 (▲), 70 (○), or 100 μM (●)]. The inset shows the plot of $t_{1/2}$ vs the reciprocal of the ticlopidine concentration for the experiments depicted.

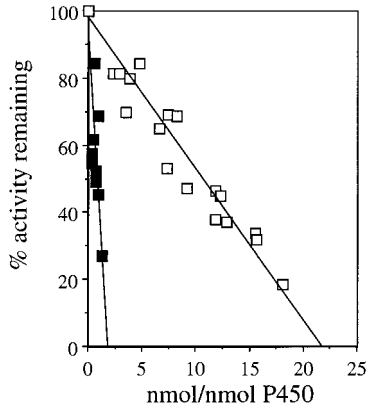


FIGURE 6: Correlation between mechanism-based P450 2C19 inactivation and efficient catalysis of ticlopidine oxidation. Microsomes from insect cells expressing CYP 2C19 were incubated under catalysis conditions in the presence of varying concentrations of ticlopidine (from 10 to 100 μM) and for different time periods (from 0 to 20 min). The amounts of metabolite **1** (□) and TSOD (■) formed, as well as the remaining P450 2C19 activity, were determined in parallel aliquots. Points are individual values from three different experiments.

Table 2) with recombinant CYP 2C19 suggests that the presence of two aryl rings, one of which is a thiophene ring, and an amine function are required for efficient binding to CYP 2C19. The loss of the *o*-chlorobenzyl substituent as in compound **2**, the loss of the amine function as in compound **3**, or the replacement of the thiophene with a benzene ring as in compounds **4** and **5** leads to a dramatic decrease of the inhibitory effects and binding ability of the corresponding compounds (Table 2).

Ticlopidine is rapidly oxidized by recombinant CYP 2C19 with formation of several metabolites. The two main metabolites have been identified by comparison with authentic samples. The major one, **1**, is derived from the 2-hydroxylation of the thiophene ring of ticlopidine. It exists under the form of the keto tautomer shown in Figure 2 and has been already detected as a ticlopidine metabolite in vivo (*58*). The second metabolite, TSOD, is a ticlopidine *S*-oxide dimer that derives from an S-oxidation of the thiophene ring of ticlopidine and a Diels−Alder type dimerization of this *S*-oxide. Evidence for the formation of thiophene *S*-oxides as intermediates in the oxidative metabolism of thiophene compounds has been previously obtained (*61*−*64*), and Diels−Alder type dimerization is a classical reaction for thiophene *S*-oxides (*64*−*67*). The formation of thiophene *S*-oxide dimers has been already observed in the in vivo and

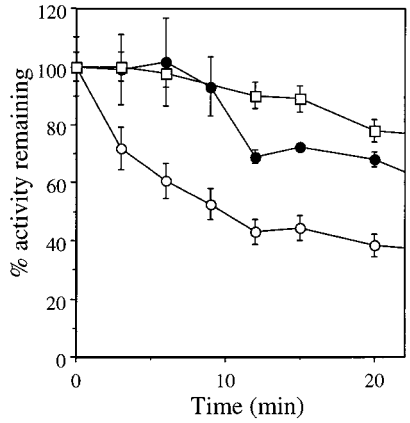


FIGURE 7: Protection by omeprazole of CYP 2C19 from ticlopidine-dependent inactivation. Microsomes from insect cells expressing CYP 2C19 were incubated with a NADPH-generating system and either with 50 μM ticlopidine (○) or with 50 μM ticlopidine plus 100 μM omeprazole (●). Control incubations (□) were performed in the presence of a NADPH-generating system and 100 μM omeprazole but without ticlopidine. Data correspond to the mean ± SD of three experiments.

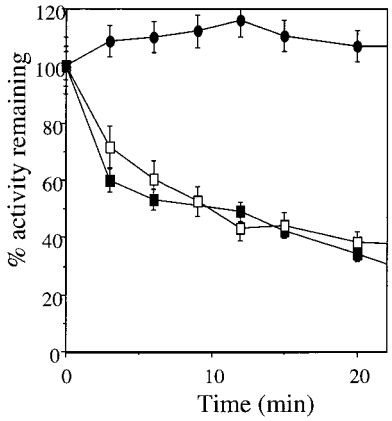


FIGURE 8: Effects of GSH on the rate of P450 2C19 inactivation by ticlopidine. Loss of P450 2C19 activity after NAPDH-dependent oxidation of 50 μM ticlopidine was evaluated as a function of time in the presence (■) and absence (□) of 5 mM GSH. Control incubations (●) containing the NADPH-generating system with GSH and without ticlopidine added were also run in parallel. Values are means ± SD from three independent experiments.

in vitro metabolism of thiophene itself (*64*). During the CYP 2C19-catalyzed oxidation of ticlopidine, CYP 2C19 undergoes an inactivation process that clearly meets the chemical and kinetic criteria generally accepted for selective, mechanism-based enzyme inactivation (*54*, *57*). This inactivation occurs in parallel with ticlopidine oxidation by CYP 2C19 in the presence of NADPH (Figure 6). It depends on ticlopidine concentration and incubation time (Figure 5). The presence of a well-known CYP 2C19 substrate, omeprazole, clearly inhibits this inactivation (Figure 7). Finally, covalent binding of ticlopidine metabolite(s) to the protein is correlated with this inactivation, the covalent binding of about 2 nmol of ticlopidine metabolite(s)/nmol of CYP 2C19 leading to an almost complete inactivation of CYP 2C19 (Figure 9). Moreover, CYP 2C19 inactivation during ticlopidine oxidation is not inhibited by the presence of 5 mM GSH in the incubation medium (Figure 8), indicating that inactivation is due to an alkylation that occurs within the active site of CYP 2C19.

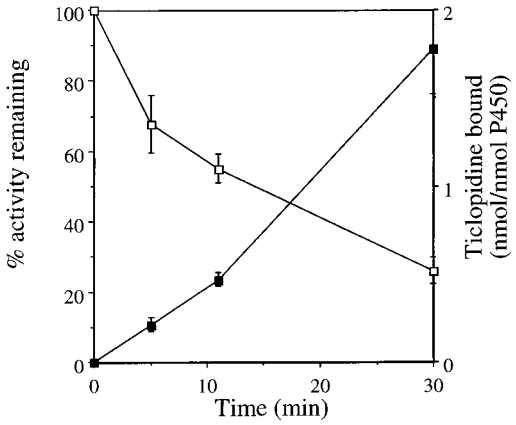


FIGURE 9: Relationship between CYP 2C19 inactivation (□) and covalent binding of ticlopidine metabolites to protein (■) as a function of time. Purified CYP 2C19 was incubated for different times in the presence of a NADPH-generating system, 100 μM [^{14}C]-ticlopidine, and 5 mM GSH, as detailed in Materials and Methods. The data shown are mean values ± SD from three separate experiments.

As shown in Figure 4, CYP 2C8 and CYP 2C9 are not inactivated during ticlopidine oxidation under conditions identical to those used for CYP 2C19. Moreover, CYP 2C18 only lost 10% of its activity after 30 min incubation in the presence of ticlopidine and NADPH under identical conditions (by comparison to control incubations without ticlopidine; data not shown). Ticlopidine thus appears as a selective mechanism-based inhibitor within the human CYP 2C subfamily. Since ticlopidine exhibits an IC_{50} value similar for inhibition of CYP 2C19 and CYP 2D6 (Table 1), preliminary experiments have been done on the oxidation of ticlopidine by CYP 2D6. Metabolites **1** and TSOD were also formed upon oxidation of ticlopidine by CYP 2D6 expressed in the W(R)fur yeast, however with rates 3−4 times smaller than in the case of CYP 2C19. Moreover, no significant loss of CYP 2D6 activity and no significant covalent binding of ticlopidine-derived species to proteins could be detected after incubation of CYP 2D6 in the presence of ticlopidine and NADPH, under conditions previously used in the case of CYP 2C19 (data not shown). These data suggest that ticlopidine could be a selective mechanism-based inhibitor of CYP 2C19, even though more detailed experiments on CYP 2D6 and additional experiments on the other main human liver CYPs [such as CYP 1A2 and 3A4, which exhibit a lower affinity for ticlopidine (Table 1)], remain to be done before drawing any definitive conclusion on that selectivity.

There is a great analogy between the behavior of ticlopidine toward CYP 2C19 and the one previously described for TA toward CYP 2C9 (*19*). Both molecules contain a thiophene ring and are mainly oxidized by CYP 2C19 or 2C9 at the level of this thiophene ring. In both cases, the major metabolite eventually derives from a hydroxylation of the thiophene moiety. Finally, in both cases, enzyme inactivation is correlated with the covalent binding of a reactive metabolite to proteins.

The mechanism previously proposed for CYP 2C9 inactivation by TA involved the intermediate formation of a very electrophilic thiophene *S*-oxide that would either evolve toward 5-hydroxy-TA or react with a nucleophilic residue of the CYP 2C9 active site (*19*). This proposition was based

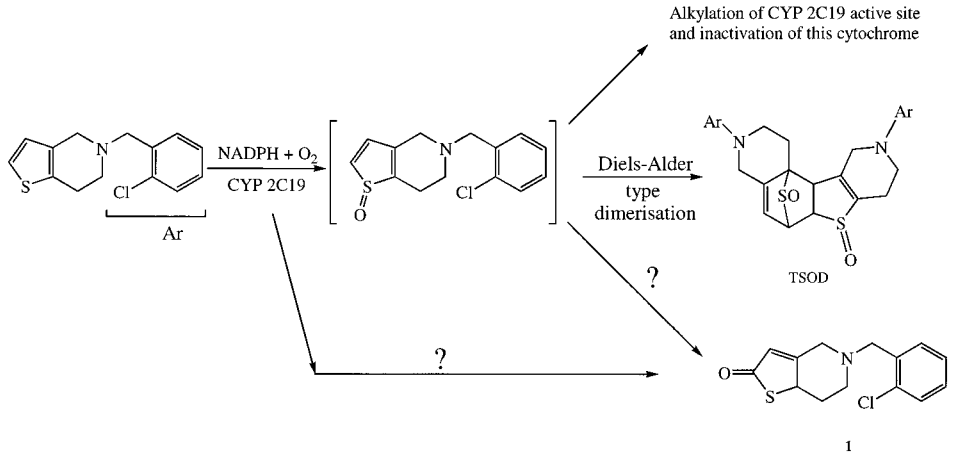


FIGURE 10: Proposed mechanism for the CYP 2C19-catalyzed oxidation of ticlopidine and the suicide inactivation of this cytochrome. This mechanism is based on the involvement of ticlopidine *S*-oxide as a key reactive intermediate in the formation of TSOD and **1** and in CYP 2C19 inactivation. However, from the presently available data, one cannot exclude the involvement of another reactive intermediate, a thiophene epoxide, in formation of **1** and CYP 2C19 inactivation.

on previously reported data on the oxidative metabolism, in vitro and in vivo, of thiophene (*62*, *64*) and of an isomer of TA (*61*, *63*) which provide evidence for thiophene *S*-oxides as key reactive intermediates in these oxidations. Actually, final, isolated metabolites of thiophene itself or of the isomer of tienilic acid are derived from two reactions of the corresponding *S*-oxide, i.e., its dimerization by a Diels−Alder-type reaction and its reaction with thiol nucleophiles, such as GSH, which leads to mercapturates or similar sulfur-containing adducts (*56*, *61*−*64*, *68*).

The CYP 2C19-catalyzed oxidation of ticlopidine mainly occurs at the level of its thiophene ring and should involve, at least in part, the intermediate formation of its *S*-oxide, as demonstrated by the presence of its *S*-oxide dimers, TSOD (Figure 2). The reactive intermediate responsible for CYP 2C19 inactivation must be formed during oxidation of ticlopidine to **1** and TSOD, as we have shown that these two metabolites do not lead by themselves to any inactivation of CYP 2C19 in the presence or in the absence of NADPH (data not shown). It is thus tempting to propose that ticlopidine-mediated inactivation of CYP 2C19 is due to the covalent binding of the reactive ticlopidine *S*-oxide metabolite to the CYP 2C19 active site. In fact, after its formation by CYP 2C19-catalyzed S-oxidation of ticlopidine, this electrophilic intermediate could either react with a nucleophilic residue of an amino acid of the CYP 2C19 active site or dimerize with formation of TSOD (Figure 10). Another possible evolution of ticlopidine *S*-oxide would be an isomerization leading to metabolite **1**. However, from the data presently available, one cannot exclude that **1** is derived from another reactive intermediate, such as an epoxide of the thiophene ring. Further experiments are necessary in order to conclude between a single reactive intermediate, ticlopidine *S*-oxide, that partitions between metabolites **1** and TSOD and leads to CYP 2C19 inactivation, and the involvement of two reactive intermediates, ticlopidine *S*-oxide and a thiophene epoxide, that lead to TSOD and **1**, respectively. Each of those reactive intermediates could be responsible for CYP 2C19 inactivation.

Table 3 compares the kinetic characteristics of ticlopidine-dependent inactivation of CYP 2C19 and TA-dependent inactivation of CYP 2C9. The $t_{1/2max}$ and k_{inact} of the two reactions are identical (3.4 min and 3.4×10^{-3} s^{-1} as mean values); they indicate that ticlopidine and TA are efficient suicide substrates when compared to known inactivators of cytochromes P450 or other enzymes (*19*, *55*, *30*−*36*). However, the K_I value found for ticlopidine is about 20 times higher than that described for TA (87 ± 30 instead of 4.3 ± 0.6 μM). This is in agreement with the difference observed in K_M values for ticlopidine and TA oxidations. The partition ratio calculated for ticlopidine-dependent inactivation of CYP 2C19 would be around 26, if one takes into account the formation of **1** + TSOD, a value markedly larger than the one reported for TA-dependent inactivation of CYP 2C9. From these data, ticlopidine appears as a little less efficient mechanism-based inhibitor than TA.

Table 3: Comparison of the Kinetic Parameters Calculated for Inactivation of CYP 2C19 by Ticlopidine (This Work) and CYP 2C9 by Tienilic Acid (*19*)

	2C19 + ticlopidine	2C9 + AT
$t_{1/2max}$ (min)	3.4 ± 1.0	3.4 ± 0.8
$k_{inact} \times 10^3$ (s^{-1})	3.2 ± 0.6	3.6 ± 0.8
K_I (μM)	87 ± 30	4.3 ± 0.6
k_{inact}/K_I ($L \cdot mol^{-1} \cdot s^{-1}$)	37 ± 15	813
K_M (μM)[a]	67 ± 10 (for TSOD) 46 ± 10 (for **1**)	5.9 ± 2.3 (for 5-OHTA)
r (partition ratio)[b]	4 ± 1 (based on TSOD) 22 ± 3 (based on **1**)	12

[a] In the case of ticlopidine oxidation, the K_M values for the formation of TSOD or of **1** are indicated; in the case of tienilic acid oxidation, the K_M value corresponds to the formation of the 5-hydroxy metabolite (*18*). [b] For CYP 2C19-catalyzed oxidation of ticlopidine, two r values can be calculated; they correspond to the number of turnovers leading to TSOD before CYP 2C19 inactivation and to the number of turnovers leading to **1** before inactivation, respectively.

However, ticlopidine is the first suicide substrate reported for CYP 2C19 and the second example of thiophene compounds acting as mechanism-based inactivators of cytochromes P450. It should be a very interesting tool to study the topology of the active site of CYP 2C19. In that context, it is noteworthy that ticlopidine, a positively charged molecule at physiological pHs, well recognizes CYP 2C19, whereas TA, a negatively charged molecule, is specific to CYP 2C9.

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