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Characterization of human cytochrome P-450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine

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Running title page

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- d) Abbreviations: CYP, cytochrome P-450; DDC, diethyldithiocarbamic acid; HPLC, high performance liquid chromatography; K_m , the Michaelis constant; KET, ketoconazole; NAPH, α -naphthoflavone; QUIN, quinidine; SULF, sulfaphenazole; TICLOP, ticlopidine; V_{max} , maximum velocity of the reaction.

Abstract

The aim of the present study was to identify human cytochrome P-450 enzymes (CYPs) involved in mono-2-, di-2- and 5-sulphoxidation, and N-demethylation of the piperidine-type phenothiazine neuroleptic thioridazine in the human liver. The experiments were performed *in vitro* using cDNA-expressed human CYPs (Supersomes 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4), liver microsomes from different donors and CYP-selective inhibitors. The results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulphoxidation and N-demethylation (34-52%), while CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulphoxidation of thioridazine in human liver (49 and 64%, respectively). Besides CYP2D6, CYP3A4 contributes to a noticeable degree to thioridazine mono-2-sulphoxidation (22%). Therefore the sulphoridazine/mesoridazine ratio may be an additional and more specific marker than the mesoridazine/thioridazine ratio for assessing the activity of CYP2D6. In contrast to promazine and perazine, CYP2C19 insignificantly contributes to the N-demetylation of thioridazine. Considering serious side-effects of thioridazine and its 5-sulphoxide (cardiotoxicity), as well as strong dopaminergic D₂ and noradrenergic α_1 receptor blocking properties of mono-2- and di-2-sulphoxides, the obtained results are of pharmacological and clinical importance, in particular in a combined therapy. Knowledge of the catalysis of thioridazine metabolism helps to choose optimum conditions (a proper co-administered drug and dosage) to avoid undesirable drug interactions.

Introduction

Thioridazine, a prototypic agent for phenothiazine neuroleptics of the piperidine-type, is a mild neuroleptic which acts on positive and negative symptoms of schizophrenia, displaying sedative and antidepressant effects. Because of its psychotropic profile, thioridazine is suitable for combination with antidepressants in the therapy of many psychiatric disorders (psychotic depression, "treatment-resistant" depression, depression in the course of schizophrenia, schizoaffective psychosis). However, the main side-effects of thioridazine are related to cardiac muscle conduction and anticholinergic activity. Among neuroleptic drugs, thioridazine produces most distinct ECG abnormalities which are dose-dependent (Axelsson 1977, Gottschalk et al., 1978; Llerena et al., 2002). Heiman (1977) reported cases of life-threatening ventricular arrhythmia in patients who had ingested a combination of thioridazine and imipramine or amitriptyline, alerting clinicians to the risk of using thioridazine at high doses and in combinations with tricyclic antidepressants. Therefore, knowledge of thioridazine metabolism seems to be of importance.

Like other phenothiazine neuroleptics, thioridazine undergoes S-oxidation in the thiazine ring in position 5, as well as aromatic hydroxylation (mainly in position 7), N-demethylation and N-oxidation (Papadopoulos et al., 1985; Svendsen et al., 1986; Lin et al., 1993). However, unlike other phenothiazines, thioridazine forms a sulphoxide in position 2 of the thiomethyl substituent (mesoridazine) which is further oxidized to a sulphone (sulphoridazine) (Figure 1). Metabolites formed by S-oxidation in position 2, i. e., mesoridazine and sulphoridazine, are more potent than thioridazine in blocking dopaminergic D₂ and noradrenergic α_1 receptors; moreover, N-desmethylthioridazine retains affinity for α_1 receptors (Axelsson, 1977; Bylund, 1981; Richelson and Nelson, 1984; Hyttel et al., 1985). Thioridazine 5-sulphoxide (a ring sulphoxide) is not pharmacologically active at

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dopaminergic or noradrenergic receptors, but is considered to contribute to the cardiotoxicity of the parent compound (Gottschalk et al., 1978; Hale and Poklis, 1986).

It is still unclear which enzymes are responsible for particular metabolic steps of thioridazine. Clinical studies demonstrated that the metabolism of thioridazine was under genetic control of hepatic CYP2D6 catalyzing 2-sulphoxidation of the neuroleptic (Meyer et al., 1990; Llerena et al., 2000, 2001). Moreover, Llerena et al. (2000) suggested that the mesoridazine/thioridazine ratio might be a useful tool to assess CYP2D6 activity. Other clinical studies showed that the plasma concentrations of thioridazine in psychiatric patients were influenced by tobacco smoking (CYP1A2 inducer) and CYP2D6 genotype, but not by the CYP2C9 genotype, which suggested that CYP1A2 in addition to CYP2D6 was involved in the metabolism of thioridazine (Berecz et al., 2003).

Thus, there are no complete data on the enzymatic catalysis of thioridazine metabolism in humans. Many other CYP enzymes which might be involved in the mono-2-sulphoxidation of thioridazine, as well as the catalysis of other metabolic pathways of this neuroleptic, such as di-2-sulphoxidation, N-demethylation and 5-sulphoxidation processes have not been studied in humans so far. Considering serious side-effects of thioridazine and the risk of its application in a combination therapy, in the present study we aimed to concurrently investigate the contribution of human CYPs to thioridazine mono-2-, di-2- and 5-sulphoxidation, and N-demethylation using different *in vitro* models.

The obtained results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulphoxidation and N-demethylation, while CYP2D6 is the key enzyme that catalyzes mono-2- and di-2-sulphoxidation of thioridazine in human liver; besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulphoxidation. The above results are compared with the findings of analogous experiments with other phenothiazine neuroleptics and are discussed in respect of the substrate-structure differences in the

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enzymatic catalysis of the metabolism of phenothiazines. Moreover, pharmacological and clinical aspects of the obtained results are emphasized.

Materials and Methods

Drugs and chemicals. Thioridazine hydrochloride was obtained from Jelfa (Jelenia Góra, Poland). Mesoridazine and sulphoridazine (free bases) were donated by Sandoz Pharma AG (Basel, Switzerland). Thioridazine 5-sulphoxide and N-desmethylthioridazine were synthesized in our laboratory as described previously (Daniel et al., 1997). α -Naphthoflavone, DDC (diethyldithiocarbamic acid), quinidine, sulfaphenazol, ticlopidine, ketoconazole and NADPH were purchased from Sigma (St. Louis, USA). All the organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

Human liver microsomes. Human liver microsome preparations (HK23, HK25, HK37, HG03, HG43, HG56, HG89, HG93) were obtained from Gentest Co. (Woburn, MA., USA).

Liver microsomes from patients HK23, HG43 and HG89 were used for optimizing the conditions of thioridazine metabolism. Microsomal protein, 250 μ g, was resuspended in 500 μ l of 20 mM TRIS/HCl buffer (pH = 7.4). To determine enzyme kinetic parameters, the thioridazine concentrations used ranged from 5 to 250 μ M. For studies of thioridazine metabolism in individual patients 25 μ M thioridazine was used. For inhibition studies, 25 μ M thioridazine was incubated with the selective CYP inhibitors: 2 μ M α -naphthoflavone (a CYP1A2 inhibitor), 200 μ M DDC (a CYP2A6 + CYP2E1 inhibitor), 10 μ M sulfaphenazole (a CYP2C9 inhibitor), 5 μ M ticlopidine (a CYP2C19 inhibitor), 10 μ M quinidine (a CYP2D6 inhibitor) and 2 μ M ketoconazole (a CYP3A4 inhibitor). After a 3-min preincubation at 37°C, the reaction was initiated by adding NADPH to a final concentration of 1 mM. After a 25-min

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incubation, the reaction was stopped by adding 200 μ l of methanol. Thioridazine and its metabolites were analyzed by an HPLC method as described below.

Correlation analysis of the data. The rates of thioridazine mono-2-, di-2- and 5-sulphoxidation, and N-demethylation were correlated with the rates of CYP-specific reactions: phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin N-demethylation (CYP2B6), diclofenac 4'-hydroxylation (CYP2C9), *S*-mephenytoin 4'-hydroxylation (CYP2C19), bufuralol 1'-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1) and testosterone 6 β -hydroxylation. Each data pair was compared by a simple linear regression analysis using the statistical programme Prism 2.01. Monooxygenase activities for each liver microsomal preparation (donor) were determined and provided by Gentest Co. (Woburn, MA., USA).

cDNA-expressed human CYPs. Microsomes from baculovirus-infected insect cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Supersomes) were obtained from Gentest Co. (Woburn, MA., USA). Thioridazine metabolism was studied under experimental conditions similar to those described for liver microsomes, with 10, 25, 50, 100 and 250 μ M thioridazine, except for the fact that the final concentration of CYPs was 100 pmol/ml. Thioridazine and its metabolites were analyzed by HPLC as described below.

Determination of thioridazine and its metabolites in the incubation medium.

Thioridazine and its metabolites were quantified using the previously described HPLC method (Daniel et al., 1997). After incubation, the samples were centrifuged for 10 min at 2000 x g. The water phase containing thioridazine and its metabolites was extracted (pH = 12)

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with hexane and 1.5% of isoamyl alcohol. The residue obtained after evaporation of the microsomal extracts was dissolved in 100 μ l of the mobile phase described below. An aliquot of 20 μ l was injected into the HPLC system. The concentrations of thioridazine and its metabolites (mesoridazine, sulphoridazine, thioridazine 5-sulphoxide, N-desmethylthioridazine) were assayed using a LaChrom (Merck-Hitachi) HPLC system with fluorescence detector. The analytical column (Econosphere C18, 5 μ m, 4.6 x 250 mm) was purchased from Alltech (Carnforth, England). The mobile phase consisted of an acetate buffer, pH=3.4 (100 mmol of ammonium acetate, 20 mmol of citric acid and 1ml of triethylamine in 1000 ml of the buffer, adjusted to pH=3.4 with an 85% phosphoric acid), and acetonitrile in the proportion 50:50. The flow rate was 1.5 ml min⁻¹, the column temperature was ambient. The fluorescence was measured at a wavelength of 270 nm (excitation) and 467 nm (emission) for thioridazine, mesoridazine, sulphoridazine and N-desmethylthioridazine, and 274/388 nm for thioridazine 5-sulphoxide. The compounds were eluted in the following order: sulphoridazine (5.49 min), mesoridazine (7.82 min), diastereoisomers of thioridazine 5-sulphoxide (9.06 min and 10.27 min), N-desmethylthioridazine (12.86 min) and thioridazine (16.81 min). The sensitivity of the HPLC method allowed us to quantify thioridazine as low as 0.004 nmol per ml ; mesoridazine and thioridazine 5-sulphoxide as low as 0.002 nmol per ml; sulphoridazine and N-desmethylthioridazine as low as 0.001 nmol per ml of a microsomal suspension. The reproducibility coefficient of variation was under 3%.

Results

Thioridazine metabolism in human liver microsomes. Figures 2A, 2B, 2C and 2D show the representative Eadie-Hofstee plots for thioridazine oxidation processes in liver microsomes from the patient HK23. All the plots were nonlinear, except for the plot for

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thioridazine di-2-sulphoxidation. Similar results were obtained with liver microsomes from patients HG43 and HG89.

Correlation study. Interindividual variability of the rates of thioridazine metabolism ranged from 2.5-fold (N-demethylation) to 3.9-fold (5-sulphoxidation) (Figure 3).

The rates of formation of thioridazine metabolites by different preparations of human liver microsomes (Figure 3) were compared with the monooxygenase activities determined for each liver preparation by the supplier (Gentest Co.). The results of those analyses are shown in Table 1, where the correlation coefficient (r) and the p value are given for each pair of data. The formation of thioridazine 5-sulphoxide and N-desmethylthioridazine was significantly correlated with phenacetin *O*-deethylase (CYP1A2) and testosterone 6 β -hydroxylase (CYP3A4) activities, while the production of thioridazine mono-2-sulphoxide (mesoridazine) and thioridazine di-2-sulphoxide (sulphoridazine) was correlated well with bufuralol 1'-hydroxylase activity (CYP2D6). Moreover, thioridazine mono-2-sulphoxidation also correlated significantly with testosterone 6 β -hydroxylase activity (CYP3A4).

No correlation was observed between the production of thioridazine metabolites and the rates of coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin N-demethylation (CYP2B6), diclofenac 4'-hydroxylation (CYP2C9), *S*-mephenytoin 4'-hydroxylation (CYP2C19) and chlorzoxazone 6-hydroxylation (CYP2E1).

Inhibition of thioridazine metabolism by CYP-selective inhibitors. α -Naphthoflavone (a CYP1A2 inhibitor) and ketoconazole (a CYP3A4 inhibitor) significantly decreased the rate of thioridazine 5-sulphoxidation (up to 52 and 62% of the control value, respectively) and thioridazine N-demethylation up to 54 and 48% of the control value, respectively) (Figure 4). On the other hand, quinidine (a CYP2D6 inhibitor) exerted a strong inhibitory effect on the

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rate of thioridazine mono-2- and di-2-sulphoxidation (up to 48 and 35% of the control value, respectively) (Figure 4). Moreover, ketoconazole significantly reduced the rate of thioridazine mono-2-sulphoxidation (up to 76% of the control value). DDC (a CYP2A6+CYP2E1 inhibitor), sulfaphenazol (a CYP2C9 inhibitor) and ticlopidine (a CYP2C19 inhibitor) had no inhibitory effect on the rate of thioridazine metabolism.

Study with cDNA-expressed human CYPs. The Lineweaver-Burk analysis of the thioridazine metabolism in cDNA-expressed human CYPs is presented in Table 2. The obtained kinetic parameters showed distinct inter-isoform differences, which was consistent with the multienzyme Eadie-Hofstee plots derived from liver microsomes (Figures 2A - 2D). The highest intrinsic clearance (V_{\max}/K_m) was found for CYP1A2 and CYP3A4 in the case of 5-sulphoxidation and N-demethylation, and for CYP2D6 in the case of mono-2- and di-2-sulphoxidation.

The ability of cDNA-expressed human CYPs to metabolize thioridazine at its low, therapeutic concentration (10 μ M) is shown in Figures 5A, 5B, 5C and 5D. According to the calculated intrinsic clearance values, the preference of CYP isoforms for catalyzing thioridazine metabolism was as follows (pmol of product/pmol of CYP isoform/min): 2D6 > 2C19 > 1A2 \approx 2B6 > 3A4 > 2C9 for mono-2-sulphoxidation, 2D6 > 2C19 > 1A2 > 3A4 for di-2-sulphoxidation, 1A2 > 3A4 > 2E1 \approx 2A6 > 2B6 > 2D6 > 2C9 > 2C19 for 5-sulphoxidation and 1A2 > 2C19 > 3A4 > 2D6 for N-demethylation. CYP2A6 and CYP2AE1 did not produce mesoridazine at a measurable amount, while sulphoridazine and N-desmethylthioridazine were not formed by CYP2A6, CYP2B6, CYP2C9 and CYP2E1.

Quantitative estimation of the contribution of CYP enzymes to the particular metabolic pathways of thioridazine. We roughly estimated the contribution of the CYP enzymes

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studied here to thioridazine mono-2-, di-2-, 5-sulphoxidation and N-demethylation in liver microsomes on the basis of the rate of those reactions in the Supersomes and the contribution of each isoform to the total CYP content in the human liver. Calculations performed at low (therapeutic) concentration of thioridazine (10 μ M) indicate that CYP2D6 is the main isoform responsible for mono-2- and di-2-sulphoxidation (48.6 and 63.65%, respectively), while CYP1A2 and CYP3A4 are the key enzymes that catalyze 5-sulphoxidation (46.5 and 34.6%, respectively) and N-demethylation (44.1 and 51.6%, respectively) of thioridazine in the human liver (Table 3). CYP3A4 contribute to a lesser degree to thioridazine mono-2-sulphoxidation (21.7%) (Table 3). The results obtained at 10 μ M and 100 μ M (data not shown) thioridazine were similar.

In the liver the amount of a metabolite formed by an individual CYP enzyme depends on both its catalytic activity with respect to the product formation and relative contribution to the total CYP content. Therefore, although the intrinsic clearance found for CYP2C19 was higher than for CYP3A4 regarding mono-2-sulphoxidation (Table 2), the calculated contribution of CYP3A4 to this reaction was considerably greater than that of CYP2C19 (Table 3), as a result of their relative amounts in the total liver CYP (30% and 1%, respectively).

Discussion

The results presented above indicate a major contribution of CYP1A2 and CYP3A4 to thioridazine 5-sulphoxidation and N-demethylation, and of CYP2D6 to its mono-2- and di-2-sulphoxidation. It is also worth stressing that CYP3A4 contributes to mono-2-sulphoxidation of thioridazine. The above final conclusion is based on our consistent results of the Eadie-Hofstee analysis (nonlinear plots suggesting multiple enzyme-catalysis), correlation and inhibition studies, and on demonstration of the ability of cDNA-expressed CYPs to

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metabolize thioridazine. It should be mentioned that the latter results (based on the average values of CYPs in the liver) are theoretical, since actual results would depend on the interindividual variability of CYPs and the diverse contribution of individual CYPs to the total content of CYP protein in the liver.

The obtained results are similar to our recent data (Wójcikowski et al., 2003, 2004) showing the main contribution of CYP1A2 and CYP3A4 to the 5-sulphoxidation (in the thiazine ring) of the aliphatic-type phenothiazine neuroleptic promazine (31 and 39%, respectively) and piperazine-type neuroleptic perazine (32 and 30%, respectively). As for promazine and perazine, the 5-sulphoxidation of thioridazine (also in thiazine ring) was mediated by CYP1A2 (46%) and CYP3A4 (34%). However, some inter-drug differences were observed in the catalysis of side chain N-demethylation. The contribution of CYP1A2 and CYP2C19 to promazine N-demethylation was similar (35 and 32%, respectively), while CYP2C19 was the main isoenzyme catalyzing perazine N-demethylation (68%). On the contrary, CYP2C19 contributed only marginally to thioridazine N-demethylation (4%). This metabolic pathway of thioridazine was mediated by CYP1A2 (44%) and CYP3A4 (52%).

The above discrepancy may stem from different chemical structures of the side-chains of the phenothiazines studied (thioridazine – piperidine side-chain, promazine – aliphatic side-chain, perazine – piperazine side-chain), which influence their access to and the interaction with catalytic sites of cytochrome P-450. The minor contribution of CYP2C19 to thioridazine N-demethylation in a side-chain might be due to strict structural requirements of the enzyme for its substrates and to limited space around the nitrogen in the side chain (compared to promazine and perazine), which hinders oxidation of the chemically vulnerable position by CYP2C19.

The results obtained in our experiment agree with clinical studies demonstrating that CYP2D6 is the key enzyme catalyzing thioridazine 2-sulphoxidation (Meyer et al., 1990;

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Llerena et al., 2000, 2001; Berecz et al. 2003) and that CYP1A2, in addition to CYP2D6, is involved in the metabolism of thioridazine (Berecz et al. 2003). However, our calculated data showed that besides CYP2D6 (49%), CYP3A4 (22%) was substantially engaged in mono-2-sulphoxidation of thioridazine. Thus, considering the higher contribution of CYP2D6 to di-2-sulphoxidation (64%) than to mono-2-sulphoxidation (49%) of thioridazine, it seems that the sulphoridazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio, proposed by Llerena et al. (2000). The above suggestion is supported by the studies of von Bahr et al. (1991) which showed that the plasma concentration of sulphoridazine was influenced by the debrisoquine hydroxylation phenotype more than was the mesoridazine level in humans. Moreover, considering an easily detectable concentration of sulphoridazine in human plasma (though one half to one quarter of the concentration of mesoridazine) (Cohen et al., 1989; Meyer et al., 1990), the simultaneous measurement of both metabolites in plasma and determination of the two markers of CYP2D6 activity is possible. It is also important to note that in the light of our results, the CYP2D6 activity indicator expressed as the sulphoridazine/mesoridazine ratio is independent (in contrast to mesoridazine/thioridazine ratio) of the inter-individual differences in expression of CYP3A4, which may vary between patients up to 60 times (Shimada et al., 1994; Hustert et al., 2001).

Our data concerning the contribution of particular cytochrome P-450 isoenzymes to the metabolism of thioridazine may have significant implications for the prediction drug-drug interactions. Thioridazine is administered to patients for months or years, very often in combinations with antidepressant, antimanic or antianxiety drugs, which engage the same CYP enzymes for their metabolism. Considering serious-side effects of thioridazine and some of its metabolites, metabolic interaction between thioridazine and other psychotropics may be of clinical importance. Metabolic interactions of this type between thioridazine and

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antidepressant drugs have been found in rats (Daniel et al., 1999; 2000) and their serious consequences were observed in humans (Heiman, 1977).

In summary, the results of present study show that (1) different structures of phenothiazine neuroleptics (mainly the structure of a side-chain) influence their interactions with catalytic sites of cytochrome P-450; (2) CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulphoxidation and N-demethylation, while CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulphoxidation of thioridazine in human liver; (3) besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulphoxidation; (4) the relative contribution of CYP2C19 to thioridazine N-demethylation is marginal; (5) the sulphoridazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio.

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Footnotes

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Legends for figures:

FIG. 1. The main metabolic pathways of thioridazine.

FIG. 2. Eadie-Hofstee plots for thioridazine mono-2-sulphoxidation (A), di-2-sulphoxidation (B), 5-sulphoxidation (C) and N-demethylation (D) in human liver microsomes. Human liver microsomes of patient HK23 (0.5 mg of protein/ml) were incubated in a 20 mM TRIS/HCl buffer (pH = 7.4) with thioridazine (5 - 250 μ M) and NADPH (1mM) for 25 min. Similar results were obtained with microsomes of patients HG43 and HG89.

FIG. 3. Interindividual variability of thioridazine metabolism in human liver microsomes. Human liver microsomes (0.5 mg of protein/ml) were incubated in a 20 mM TRIS/HCl buffer (pH = 7.4) with thioridazine (25 μ M) and NADPH (1mM) for 25 min. Each bar represents the mean value \pm s.d. of five determinations from independent incubation.

FIG. 4. Effect of CYP-selective inhibitors on the rate of thioridazine metabolism in pooled human liver microsomes (HK23, HK25, HK37, HG03, HG43, HG56, HG89, HG93). Microsomes were incubated with 25 μ M thioridazine, in the absence (control) or presence of CYP-specific inhibitors: 2 μ M α -naphthoflavone (NAPH), 200 μ M diethyldithiocarbamic acid (DDC), 10 μ M sulfaphenazole (SULF), 5 μ M ticlopidine (TICLOP), 10 μ M quinidine (QUIN) or 2 μ M ketoconazole (KET). Absolute control values were 56.9 ± 13.7 pmol of mesoridazine/mg protein/min, 4.8 ± 0.8 pmol of sulphoridazine/mg protein/min, 38.7 ± 2.3 pmol of thioridazine 5-sulphoxide/mg protein/min and 16.0 ± 2.3 pmol of N-desmethylthioridazine/mg protein/min. Mean values \pm s.d. (n = 5) are presented. Statistical significance was assessed using Student's *t*-test and indicated with *** $p < 0.001$ and * $p < 0.05$. For further explanation see Figure 3.

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FIG. 5. Biotransformation of thioridazine via mono-2-sulphoxidation (A), di-2-sulphoxidation (B), 5-sulphoxidation (C) and N-demethylation (D) by the cDNA-expressed human CYPs (Supersomes). Thioridazine (10 μ M) was incubated with Supersomes (100 pmol of CYP/ml) and NADPH (1mM) for 25 min. Each bar represents the mean value \pm s.d. of three determinations from independent incubations.

TABLE 1

A correlation (r value) between the rate of thioridazine metabolism and the velocity of CYP- specific reactions in human liver microsomes.

| | Phenacetin <i>O</i> -deethylation (CYP1A2) | Coumarin 7-hydroxylation (CYP2A6) | <i>S</i> -mephenytoin N-demethylation (CYP2B6) | Diclofenac 4'-hydroxylation (CYP2C9) | <i>S</i> -mephenytoin 4'-hydroxylation (CYP2C19) | Bufuralol 1'-hydroxylation (CYP2D6) | Chlorzoxazone 6-hydroxylation (CYP2E1) | Testosterone 6 β -hydroxylation (CYP3A4) |
|---|--|---|--|--|--|---|--|--|
| Thioridazine mono-2- sulphoxidation | -0.21 ^{ns} | 0.58 ^{ns} | -0.50 ^{ns} | 0.20 ^{ns} | -0.52 ^{ns} | 0.74* | 0.47 ^{ns} | 0.71* |
| Thioridazine di-2- sulphoxidation | -0.37 ^{ns} | -0.06 ^{ns} | -0.90 ^{ns} | 0.05 ^{ns} | -0.25 ^{ns} | 0.73* | 0.18 ^{ns} | -0.57 ^{ns} |
| Thioridazine 5- sulphoxidation | 0.77* | 0.08 ^{ns} | 0.09 ^{ns} | -0.01 ^{ns} | 0.24 ^{ns} | -0.37 ^{ns} | 0.08 ^{ns} | 0.71* |
| Thioridazine N- demethylation | 0.72* | 0.35 ^{ns} | 0.70 ^{ns} | -0.01 ^{ns} | 0.16 ^{ns} | 0.25 ^{ns} | 0.16 ^{ns} | 0.84** |

Each pair of data was compared by a simple linear regression analysis using the statistical programme Prism 2.01. Statistical significance was indicated with **p<0.01 and *p<0.05, ns (p>0.05) – statistically not significant.

TABLE 2

Kinetic parameters of thioridazine metabolism in cDNA-expressed human CYPs (Supersomes).

| CYPs | Thioridazine mono-2-sulphoxidation | | | Thioridazine di-2-sulphoxidation | | | Thioridazine 5-sulphoxidation | | | Thioridazine N-demethylation | | |
|----------------|------------------------------------|-------------|---------------|----------------------------------|-----------|---------------|-------------------------------|-----------|---------------|------------------------------|-----------|---------------|
| | K_m^a | V_{max}^b | V_{max}/K_m | K_m | V_{max} | V_{max}/K_m | K_m | V_{max} | V_{max}/K_m | K_m | V_{max} | V_{max}/K_m |
| CYP1A2 | 160 | 1.786 | 0.0112 | 16 | 0.020 | 0.0012 | 80 | 7.519 | 0.0940 | 36 | 0.403 | 0.0112 |
| CYP2A6 | 323 | 0.485 | 0.0015 | 48 | 0.004 | 0.0001 | 34 | 0.879 | 0.0258 | 200 | 0.200 | 0.0010 |
| CYP2B6 | 67 | 0.605 | 0.0090 | 55 | 0.006 | 0.0001 | 71 | 1.412 | 0.0199 | 55 | 0.124 | 0.0022 |
| CYP2C9 | 45 | 0.221 | 0.0049 | 91 | 0.009 | 0.0001 | 114 | 1.250 | 0.0110 | 20 | 0.057 | 0.0028 |
| CYP2C19 | 47 | 6.289 | 0.1338 | 26 | 0.141 | 0.0054 | 800 | 6.993 | 0.0087 | 400 | 2.128 | 0.0053 |
| CYP2D6 | 62 | 16.667 | 0.2688 | 10 | 0.468 | 0.0468 | 385 | 5.263 | 0.0137 | 67 | 0.233 | 0.0035 |
| CYP2E1 | 333 | 0.527 | 0.0016 | 38 | 0.004 | 0.0001 | 50 | 1.160 | 0.0232 | 83 | 0.109 | 0.0013 |
| CYP3A4 | 42 | 0.302 | 0.0072 | 20 | 0.009 | 0.0004 | 28 | 1.193 | 0.0426 | 21 | 0.148 | 0.0070 |

^a K_m expressed in μM , ^b V_{max} expressed in pmol/mg protein/min

TABLE 3

Estimation of the contribution of CYP isoforms to the particular metabolic pathways of thioridazine (10 μ M) on the basis of the rates of these reaction in Supersomes and average CYPs' contents in the liver.

| CYPs | Relative contribution of the isoform to the total CYP contents in liver microsomes [fraction] * | Relative contribution of the isoform to thioridazine metabolism in liver microsomes [%] ** | | | |
|---------|---|--|----------------------------------|-------------------------------|------------------------------|
| | | Thioridazine mono-2-sulphoxidation | Thioridazine di-2-sulphoxidation | Thioridazine 5-sulphoxidation | Thioridazine N-demethylation |
| CYP1A2 | 0.127 ^(a) | 10.8 | 16.7 | 46.5 | 44.1 |
| CYP2A6 | 0,040 ^(a) | n.d. | n.d. | 3.5 | n.d. |
| CYP2B6 | 0.002 ^(a) | 0.2 | n.d. | 0.2 | n.d. |
| CYP2C9 | 0.180 ^(b) | 6.6 | n.d. | 8.2 | n.d. |
| CYP2C19 | 0.010 ^(b) | 12.1 | 6.1 | 0.4 | 2.7 |
| CYP2D6 | 0.015 ^(a) | 48.6 | 63.6 | 0.9 | 1.6 |
| CYP2E1 | 0.066 ^(a) | n.d. | n.d. | 6.0 | n.d. |
| CYP3A4 | 0.288 ^(a) | 21.7 | 13.6 | 34.3 | 56.1 |

* Data according to Shimada *et al.* (1994)^(a) and Lewis (2002)^(b).

** Relative contribution of CYPs to the particular metabolic pathways of thioridazine was calculated as percentage of the sum of predicted velocities in liver microsomes. The predicted velocity in liver microsomes was calculated by multiplying the velocity in Supersomes (see Figure 5) by the relative contribution of isoform to the total CYP content in liver microsomes. For details see Wójcikowski *et al.* (2003, 2004).

n.d. – not detected

Figure 1

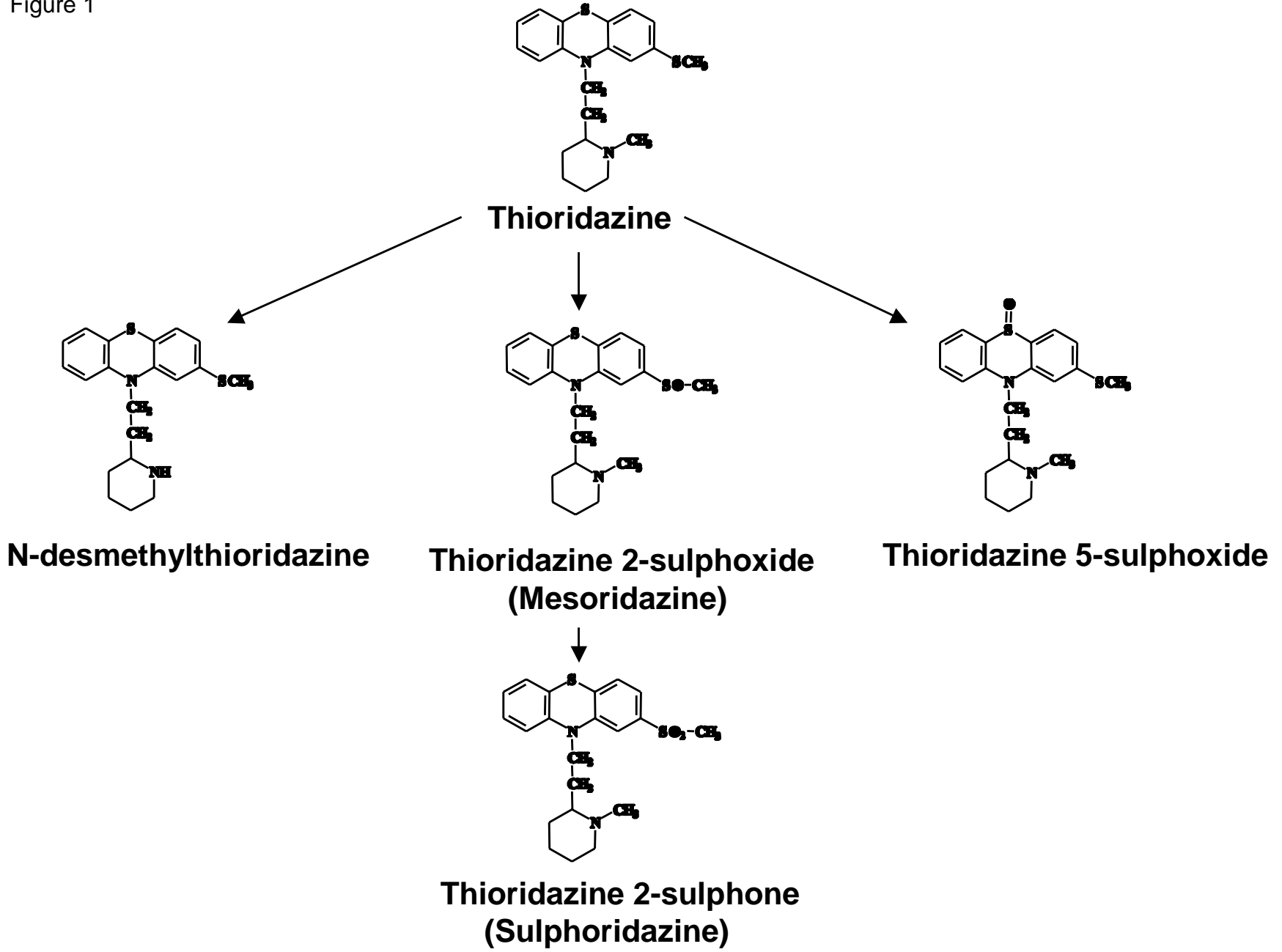


Figure 2

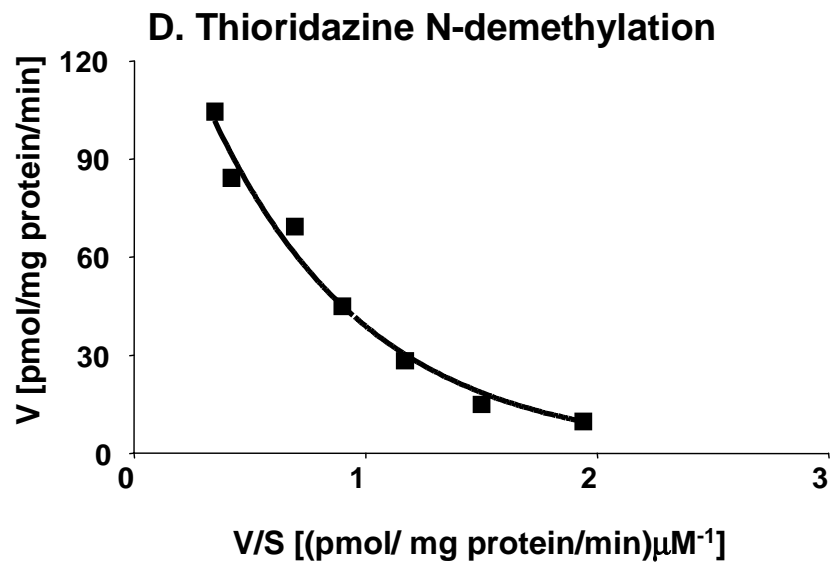
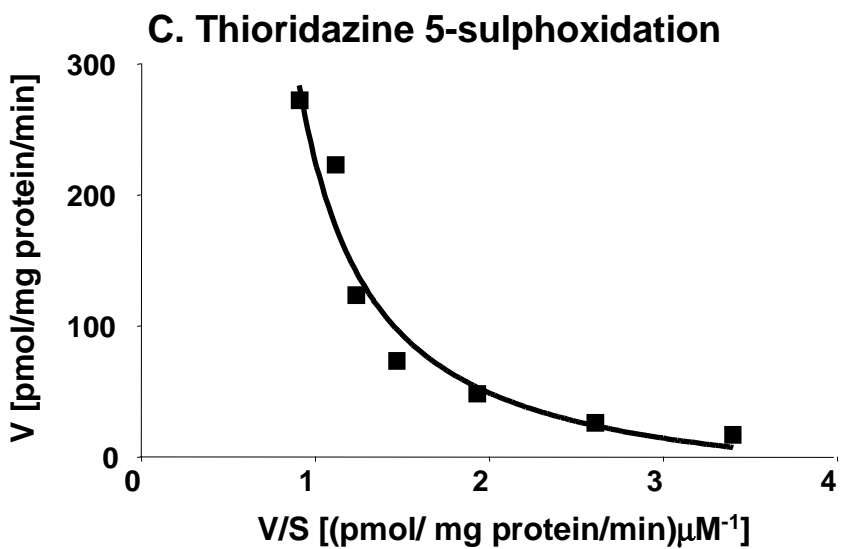
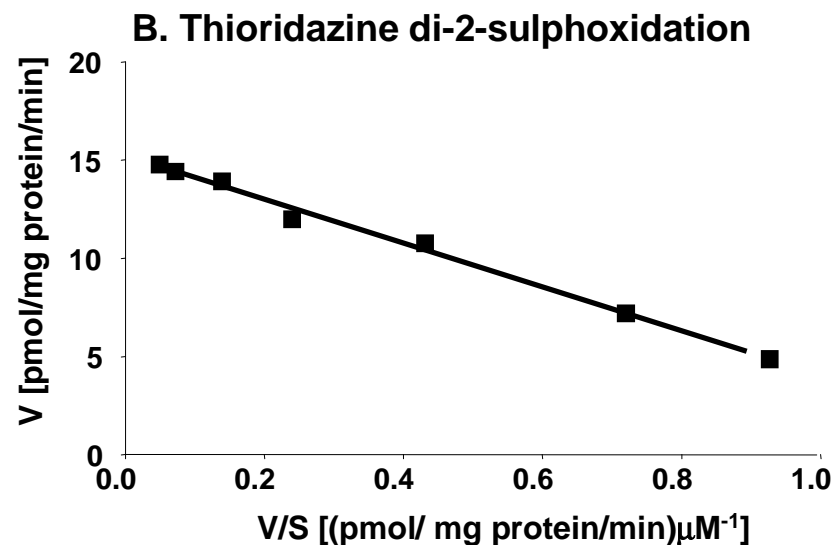
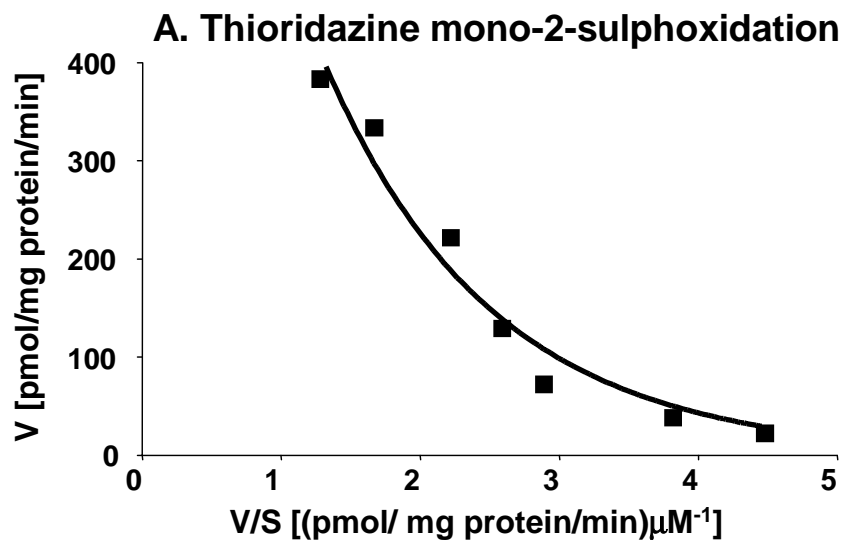


Figure 3

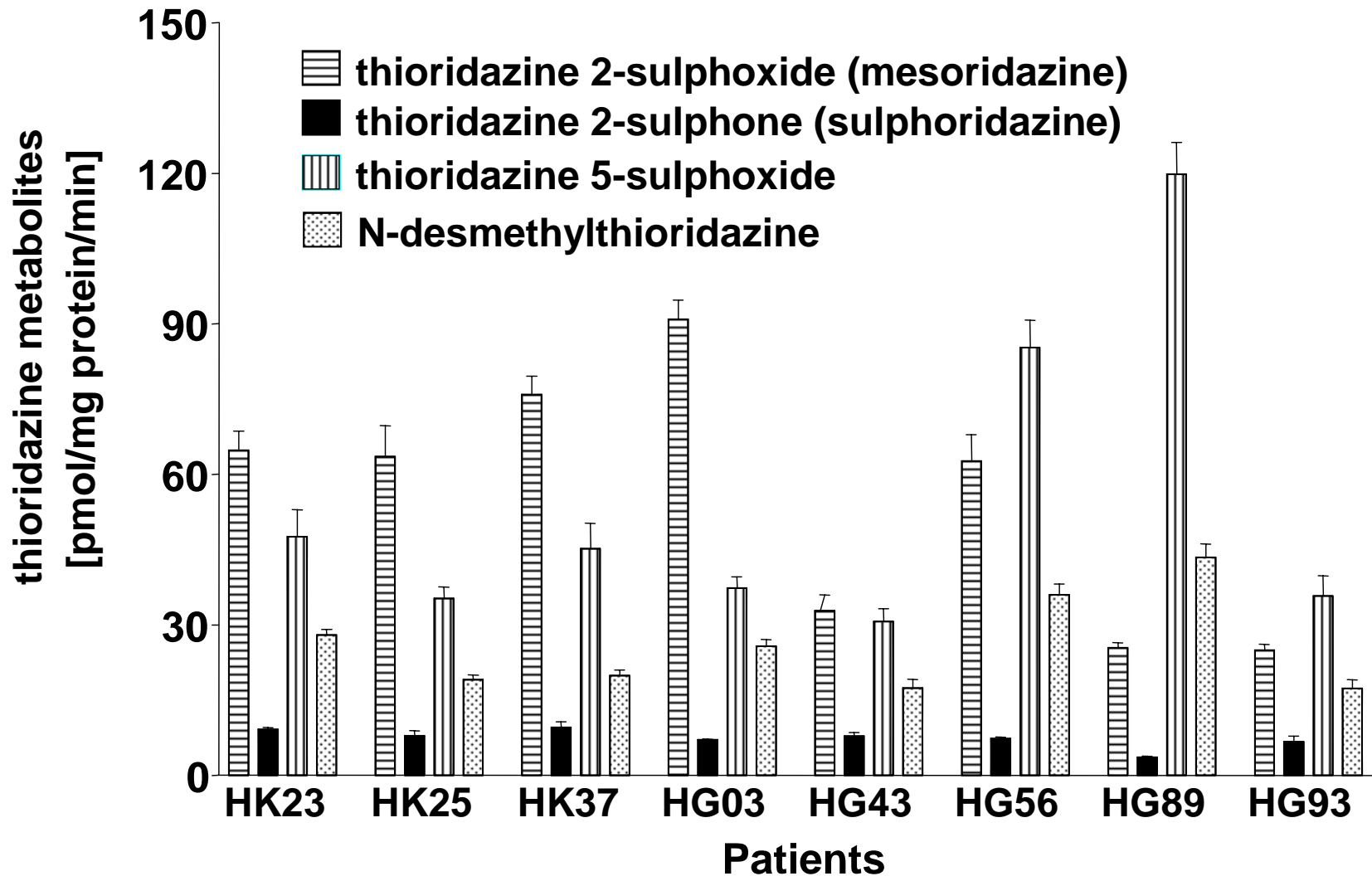


Figure 4

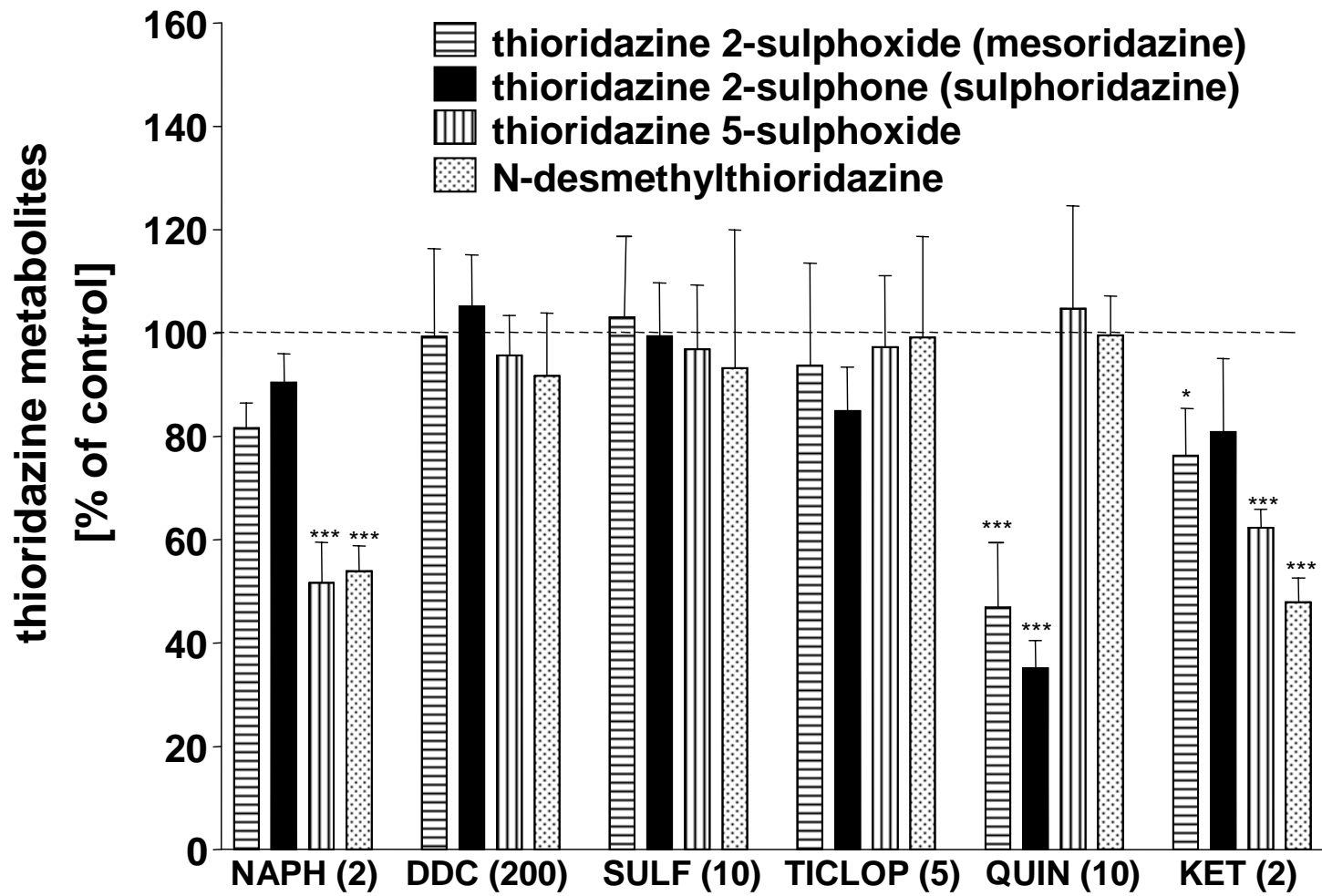


Figure 5

