Effect of Guaiazulene on Some Cytochrome P450 Activities. Implication in the Metabolic Activation and Hepatotoxicity of Paracetamol ☆

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Key Words: *Guaiazulene; drug metabolism; paracetamol; cytochrome P450*

Summary

The *in vitro* and *in vivo* effect of guaiazulene, a natural azulene derivative, on rat hepatic cytochrome P450 (CYP) is investigated. Furthermore, paracetamol hepatotoxicity is induced in rats and the activity of specific cytochrome P450 forms, involved in the metabolic activation of paracetamol to the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) is examined, after the administration of guaiazulene, using diagnostic cytochrome P450 substrates. It is found that guaiazulene inhibited considerably CYP1A2 and CYP2B1 and had a weak effect on CYP1A1 in rat hepatic microsomal fractions. Guaiazulene administered to rats did not produce any macroscopic toxic effect and caused no change of liver weight, microsomal protein and total cytochrome P450 content. Guaiazulene inhibited CYP1A2 activity in rats with or without paracetamol intoxication. Considering that CYP1A2 participates in the formation of NAPQI, as well as in the metabolic activation of several toxic and carcinogenic compounds, these results, in combination with the antioxidant activity of guaiazulene that we have found in previous investigations, indicate potential useful applications of guaiazulene.

Introduction

The cytochrome P450 (CYP) superfamily constitutes the major xenobiotic-metabolizing enzyme system and the importance of these enzymes in drug and endogenous compound metabolism, toxicology, and carcinogenesis is well established. Work on cloning and expression of cytochromes P450 have resulted in the characterization of more than 300 prokaryotic and eukaryotic cytochrome P450 forms [1]. The activities of several CYP subsets can be modulated in response to certain factors, such as stress [2], diet, disease states, exposure to drugs and other chemicals, or oxidative stress [3]. In humans, members of the CYP1, CYP2, and CYP3 families, primarily involved in the hepatic metabolism of xenobiotics, are known to be up- or down-regulated by a number of endogenous or exogenous agents [4]. Furthermore, the activation of molecular oxygen by cytochrome P450 for the oxidation of the substrate and NADPH makes this enzyme system a potentially significant source of reactive oxygen intermediates. However, the latter reactions normally occur in a caged process, in which the reactive oxygen species are not allowed to be released into the surrounding medium [5]. This is achieved via the defense mechanism of the organisms, where the tripeptide thiol glutathione plays a major role.

The analgesic drug paracetamol, at high doses, is known to lead to glutathione depletion and to cause life-threatening kidney and especially liver damage. Paracetamol hepatotoxicity is due to its reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) and is mediated by covalent binding of NAPQI to essential functional proteins, as well as through the oxidizing ability of this metabolite [6].

In previous investigations, we have studied the antioxidant effect of the azulene derivatives chamazulene and guaiazulene, constituents of the oil of *Matricaria chamomilla*, *Asteraceae*, and *Guajacum officinalle*, *Zygophyllaceae*. It was found that chamazulene [7] and guaiazulene (unpublished results) inhibited very significantly the *in vitro* peroxidation of rat hepatic microsomal membrane lipids. In addition, guaiazulene (Fig. 1) was found to offer protection to rats against paracetamol intoxication, completely inhibiting hepatic glutathione depletion. Therefore, it appeared interesting to investigate the effect of guaiazulene on the hepatic drug metabolizing enzymes and, in particular, those cytochrome P450 members which are involved in paracetamol metabolism, in an attempt to elucidate the mechanism of the hepatoprotective action of guaiazulene.

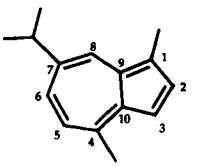

Fig 1. The chemical structure of guaiazulene.

Results

The addition of guaiazulene to the hepatic microsomal suspension from untreated (metabolism of 7-ethoxy- and 7-methoxy- resorufins) or phenobarbital-pretreated (7-pentoxy-resorufin) rats resulted in a concentration-dependent inhibition of the alkoxyresorufin *O*-dealkylation reactions. Results are shown in Figure 2. From these results, the IC_{50} values of guaiazulene for ethoxy-resorufin *O*-deethylation was found to be 5.0 mM, for methoxy-resorufin *O*-demethylation, 0.8 mM and for pentoxy-resorufin *O*-dependentylation the corresponding IC_{50} value was 0.3 mM.

The treatment of rats with paracetamol, guaiazulene or their combination had no effect on liver weight, microsomal protein and total cytochrome P450 content (Table 1). Administration of paracetamol alone increased 4-nitrophenol hydroxylation (Table 2), without affecting any other of the examined metabolic reactions. Guaiazulene treatment reduced significantly the *O*-demethylation of methoxy-re-

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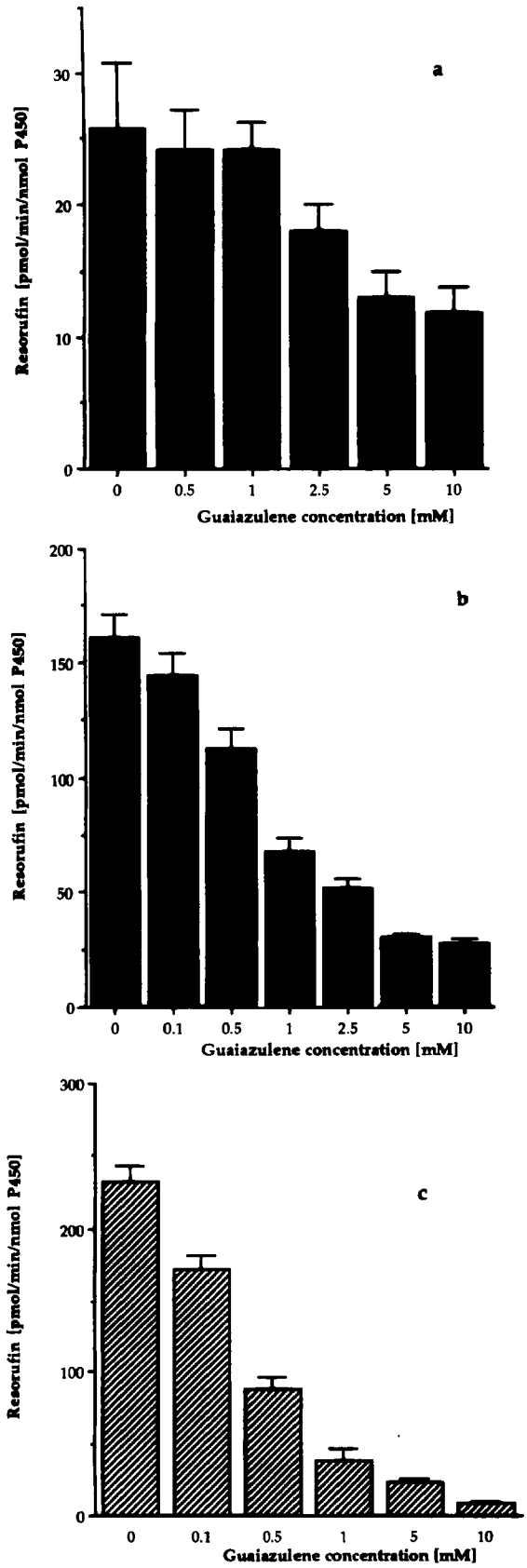

Fig 2. *In vitro* inhibition by guaiazulene of 7-alkoxyresorufin *O*-dealkylation: **a** Ethoxyresorufin (CYP1A1); **b** Methoxyresorufin (CYP1A2); **c** Pentoxyresorufin (CYP2B1).

Table 1. Effect of paracetamol and guaiazulene treatment of rats on some hepatic parameters. Results are presented as means ± SD ($n \geq 3$).

Treatment	Liver weight (g/100 g body weight)	Microsomal protein (mg/g liver)	Total cytochrome P450 (nmol/mg protein)
Control	3.65 ± 0.10	22.06 ± 1.61	0.36 ± 0.04
Paracetamol	3.65 ± 0.16	20.69 ± 2.49	0.38 ± 0.03
Guaiazulene	3.66 ± 0.15	21.01 ± 3.65	0.32 ± 0.02
Paracetamol plus Guaiazulene	3.79 ± 0.25	21.76 ± 1.84	0.32 ± 0.03

sorufin (Table 2), while no alterations in the other examined cytochrome P450 activities were observed. The above effects of paracetamol on nitrophenol hydroxylation, as well as of guaiazulene on methoxy-resorufin demethylation also appeared after the combined treatment of rats with these agents.

Discussion

The cytochrome P450 enzyme system plays a major role in the therapeutic and toxic effects of drugs. Knowledge about the dominant cytochrome P450 form responsible for the metabolism of a particular drug is valuable in predicting factors such as the efficacy of the drug or potential drug interactions.

Paracetamol is considered to be a safe drug when used at the recommended dose; however, in certain situations or after acute overdosage, paracetamol can be lethal. The drug of choice to prevent paracetamol toxicity is *N*-acetyl-L-cysteine, effective if administered 8–10 hours following paracetamol ingestion [8].

The hepatotoxicity of paracetamol is associated with its reactive metabolite NAPQI which, in humans, is produced mainly by CYP2E1, CYP1A1, CYP1A2, and CYP3A4. CYP2E1 is the primary enzyme involved at relatively low doses, while at higher doses, CYP1A1, CYP1A2, and CYP3A4 also contribute to the metabolic activation of paracetamol. The human CYP3A4 is orthologous to rat CYP3A1 and CYP3A2 [6], two forms that share 89% amino acid similarity. Although the pattern of paracetamol metabolism differs in various species, Fischer-344 rats are reported to be sensitive to paracetamol-induced hepatotoxicity [9].

NAPQI is the final product following a hydrogen abstraction and a subsequent amide radical formation. Hydrogen abstraction to form a phenolic radical, which gives the corresponding catechol metabolite, is a metabolic route catalysed by CYP2B1. Although the catechol derivative is the main metabolite through this route, a second hydrogen radical abstraction may occur at the amide group, which leads again to NAPQI as the end product, a reaction that has been shown to happen [10]. Thus, it has been reported that induction of CYP2B1/2B2 enzymes in the normal rat contributes to enhanced paracetamol hepatotoxicity due to NAPQI formation [11].

The administration of guaiazulene to rats did not cause any macroscopically detectable toxic effect under our experimental setting. In addition, guaiazulene, alone or in combination with paracetamol, did not affect the metabolism of ethoxy-re-

Table 2. Effect of paracetamol and guaiazulene treatment of rats on some hepatic cytochrome P450 activities. Results are presented as means ± SD ($n \geq 3$).

Cytochrome P450 activities	CYP	Control	Paracetamol	Guaiazulene	Paracetamol plus guaiazulene
Ethoxyresorufin *O*-deethylase pmol/min/mg prot.	1A1	18.39 ± 3.80	17.40 ± 1.15	25.55 ± 5.90	22.00 ± 2.90
Methoxyresorufin *O*-demethylase pmol/min/mg prot.	1A2	52.21 ± 2.65	49.18 ± 1.66	30.33 ± 5.30 ** a	25.80 ± 2.83 ** a
Pentoxyresorufin *O*-depentylase pmol/min/mg prot.	2B1	4.38 ± 0.62	4.98 ± 0.56	5.46 ± 1.96	4.14 ± 0.52
4-Nitrophenol hydroxylase nmol/min/mg prot.	2E1	0.77 ± 0.31	1.51 ± 0.26 * b	1.00 ± 0.28	1.64 ± 0.02 * b
Erythromycin *N*-demethylase nmol/min/mg prot.	3A1	3.39 ± 0.73	2.36 ± 0.63	2.71 ± 0.51	2.38 ± 1.06

[a] Compared with the control and the paracetamol-treated rats

[b] Compared with the control group

* $p < 0.05$, ** $p < 0.005$, Student's t-test

sorufin, considered to be a diagnostic substrate for CYP1A1[12]. When guaiazulene was added to the hepatic microsomal suspension from untreated rats, it appeared to act as a weak inhibitor of ethoxy-resorufin metabolism. However, the IC_{50} value of 5 mM calculated for this reaction is too high to indicate any significant inhibition *in vivo*.

Guaiazulene inhibited, *in vitro* and *in vivo*, the metabolism of methoxy-resorufin, specifically catalyzed by CYP1A2 [13], an isoform well conserved in mammals, responsible for the metabolic activation of several procarcinogens and promutagens, such as aflatoxin B1 or heterocyclic arylamine food pyrolysates [14]. Therefore, CYP1A2 regulation may play an important role in carcinogenic activation.

CYP1A2 was unaffected by the administration of paracetamol alone, but the inhibition offered by guaiazulene continued to be observed after the combined treatment with paracetamol and guaiazulene. Considering that CYP1A2 participates in the formation of NAPQI, it can be concluded that the inhibition offered by guaiazulene contributes to the hepatoprotective action of this natural product. It is known that a successful way of preventing paracetamol-induced hepatotoxicity is by inhibiting cytochrome P450 enzymes responsible for its metabolic activation [15]. Another approach is the prevention of glutathione depletion. We have found recently that guaiazulene administered to rats completely prevented glutathione depletion due to paracetamol intoxication, and we attributed this to the ability of guaiazulene to act as a chain-breaking antioxidant, thus inhibiting very significantly lipid peroxidation. Cytochrome P450, like other redox systems, being involved in the reduction and activation of molecular oxygen, is capable of producing reactive oxygen species and free radicals. However, under normal conditions, cytochrome P450 homeostasis is preserved, among other factors, by glutathione, a fundamental source of intracellular reducing capacity.

It has been found that cytochrome P450 degradation due to lipid peroxidation varies among the different enzymes, and suggested that the reported high degree of the CYP1A2 contribution to lipid peroxidation could be explained by the ability of this form to act as a lipid peroxidation initiator [16]. Therefore, we can conclude that the protection offered by guaiazulene against paracetamol toxicity is a beneficial combination of CYP1A2 inhibition, resulting in reduced metabolic activation of paracetamol, and the ability of guaiazulene to act as a chain-breaking antioxidant, preventing both cytochrome P450 degradation and NAPQI-induced glutathione depletion.

Guaiazulene was found to inhibit pentoxy-resorufin *O*-depentylation, catalyzed mainly be CYP2B1 [12], when added to the microsomes from phenobarbital-pretreated rats. However, it had no effect on this biotransformation when given to rats, alone or after paracetamol administration. The observed *in vitro* reduction of pentoxy-resorufin *O*-depentylation could be considered as a competition between guaiazulene and pentoxy-resorufin for the same active site of the enzyme. It appears conceivable that guaiazulene is a good ligand for the CYP2B enzymes.

It is known that any two alternate substrates for the same enzyme exhibit competitive inhibition, depending on the relative affinities of the enzyme for the two compounds and the concentrations achieved at the active site. Concerning *in vivo-in vitro* correlations with a competitive inhibitor, it is established [17] that this kind of inhibition depends on the continuous presence of the compound and is rarely observed

with washed liver microsomes from animals treated *in vivo*. Further kinetic experiments are under way for verifying the nature of the observed interaction of guaiazulene with this isoenzyme.

In a quantitative structure-activity relationship study of 10 substituted toluene derivatives which are CYP2B substrates, various molecular parameters were correlated with the affinity of these compounds to cytochrome P450 [18]. It was found that only lipophilicity and molecular volume, representing the ability of the compounds to desolvate and occupy the binding site, gave significant relationships with binding affinity. The best ligand in that series was 4-*t*-butyl-toluene, followed by 4-*iso*-propyl-toluene, a compound that possesses apparent structural similarities with guaiazulene. We calculated the CLOGP value of 4-*iso*-propyl-toluene, which was found to be 4.07, almost identical with the reported [18, 19] experimental log *P* value of 4.10, used in the aforementioned study, while the CLOGP of guaiazulene was calculated to be 5.74. The molecular volumes of 4-*iso*-propyl-toluene and guaiazulene, corresponding to the volume of the van der Waals contact surface of the molecules, were derived by Molecular Orbital calculations (AM1) and found to be 92.34 $Å^3$ and 128.39 $Å^3$, respectively. Since 4-*t*-butyl-toluene has a log *P* of 4.59 and molecular volume 118.31 $Å^3$, and considering the physicochemical and structural similarities of guaiazulene with these molecules, we can suggest that guaiazulene possesses the required structural characteristics to interact with CYP2B.

4-Nitrophenol is a selective marker for CYP2E1 [20], an enzyme primarily involved in NAPQI formation. In our experiments, paracetamol, administered alone or with guaiazulene, increased CYP2E1 metabolic activity. CYP2E1, apart from participating in the metabolism of ketone bodies during gluconeogenesis [21], is an important xenobiotic metabolizing enzyme, which demonstrates selectivity mainly for low molecular weight compounds, like ethanol, acetone, pyrazol, carbon tetrachloride. Guaiazulene was found not to influence CYP2E1 activity. Similarly, guaiazulene, administered to rats with or without paracetamol intoxication, had no effect on erythromycin *N*-demethylation, a CYP3A1-catalyzed reaction [22]. The CYP3A subfamily has an affinity for large, non-polar substrates, such as steroids or the macrolide antibiotics. Consequently, the lack of effect of guaiazulene on CYP2E1 and CYP3A1 activities, as expressed by the unaffected metabolism of 4-nitrophenol and erythromycin, respectively, with or without the concomittant administration of paracetamol, could be explained by the absence of affinity of guaiazulene for the corresponding isoforms, since it does not fulfill the structural characteristics encountered in the majority of their ligands.

Although guaiazulene could not inhibit CYP2E1 or CYP3A1, both involved in NAPQI formation and glutathione depletion, it did protected rats against paracetamol toxicity and glutathione loss. Therefore, we suggest that the hepatoprotective effect of guaiazulene is mainly a result of its antioxidant and radical scavenging ability, indirectly influencing the metabolic activation. Similar results have been reported by others, concerning the activity of curcumin [22], and by us [13] in a recent study of piperidine derivatives with strong antioxidant activity, which protected rat hepatocytes against paracetamol-induced lactate dehydrogenase leakage, without inhibiting CYP2E1 and CYP3A1 activities.

However, in the overall hepatoprotective effect of guaiazulene against paracetamol intoxication, the significant inhibition of CYP1A2 activities, observed both *in vivo* and *in vitro*, may play an additional, significant role, by decreasing the formation of the hepatotoxic metabolite. Furthermore, considering that guaiazulene is a non toxic, natural product, and that CYP1A2 is implicated in the metabolic activation of many potentially toxic substances, our results may indicate further useful applications of guaiazulene.

Experimental Part

Materials

Guaiazulene (1,4-dimethyl-7-isopropylazulene), 7-methoxy-, 7-ethoxy-, and 7-pentoxy-resorufins, 4-nitrophenol, erythromycin, phenobarbital, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Paracetamol (4-hydroxyacetanilide) was from Merck (Darmstadt, Germany). All other reagents used were of the highest commercially available purity.

Animals and Animal Treatment

Female Fischer-344 rats (180–200 g), housed in temperature (23 °C) and light (12-h lighting cycle) controlled rooms, having free access to standard laboratory chow and tap water, were used.

For the *in vitro* experiments, livers from untreated or phenobarbital-pretreated (90 mg/kg, intraperitoneally, once daily for two days) rats were excised (in the latter case, 24 h after the last pretreatment), weighed, the microsomal fraction, corresponding to 250 mg liver/ml, was prepared as described before [23] and stored at –80 °C until use.

For the *in vivo* experiment, rats were divided into four groups and treated as follows:

Group 1 received paracetamol, 600 mg/kg, orally, as a micronized aqueous suspension with few drops of Tween-80, once.

Group 2 received guaiazulene, 250 mg/kg, intraperitoneally, as a micronized aqueous suspension with few drops of Tween-80, once.

Group 3 received paracetamol as group 1. One hour later, guaiazulene was administered as in group 2.

Group 4 served as the control group, receiving only the liquid vehicle.

The experiment was terminated 18 h after the administration of paracetamol, livers were excised, weighed, the microsomal fraction was prepared individually and stored at –80 °C until use.

In Vitro Effect of Guaiazulene on Cytochrome P450-Mediated Drug Metabolism

Using the microsomal fraction from phenobarbital-pretreated or untreated rats, the *in vitro* effect of guaiazulene on the *O*-dealkylation of 7-pentoxy-resorufin or 7-ethoxy- and 7-methoxy-resorufin, respectively, was estimated as described [12, 13].

Briefly, in a fluorimetric cuvette containing 50 µl of the microsomal suspension in 2 ml of 0.1 mM Tris-HCl buffer (pH 7.8) and 3–5 µl of guaiazulene dissolved in dimethyl sulfoxide (final concentrations 0.1–10 mM), 5 µl of 1.0 mM pentoxy-resorufin or 3 µl of 0.53 mM ethoxy-resorufin or 5 µl of 0.7 mM methoxy-resorufin were added. A baseline was recorded (510 nm excitation, 586 nm emission) prior to the initiation of the reaction by the addition of 10 µl of NADPH (50 mM in 1% w/v solution of $NaHCO_3$). The reaction was monitored continously for 4 min. The instrument was calibrated using aliquots (10 µl) of resorufin (1 mM). Each experiment was performed at least in duplicate.

Effect of the Administration of Guaiazulene to Rats with Paracetamol-Induced Liver Damage on Cytochrome P450-Mediated Drug Metabolism

The hepatic microsomal fractions prepared from groups 1–4 were used for the investigation of the metabolism of the three 7-alkoxyresorufins following

the method described above (buffer was added in the place of guaiazulene). In addition, the aromatic hydroxylation of 4-nitrophenol and the *N*-demethylation of erythromycin were examined.

For the hydroxylation of 4-nitrophenol, the incubation mixture contained 50 µl of the microsomal suspension, 0.1 ml of ascorbic acid (1 mM), 0.1 ml of 4-nitrophenol (1 mM), in 0.2 M phosphate buffer pH 6.8. The reaction was started by the addition of 0.1 ml NADPH (10 mM) and terminated, after the incubation of the mixture at 37 °C for 10 min, by the addition of 0.5 ml of ice-cold 0.6 N perchloric acid. The produced nitrocatechol was estimated spectroscopically at 536 nm [20, 24].

For the *N*-demethylation of erythromycin [20, 25], the incubation mixture contained 0.1 ml of the microsomal suspension, 0.1 ml of erythromycin (10 mM) and 0.1 ml magnesium chloride solution (150 mM) in 50 mM phosphate buffer pH 7.25. The reaction was initiated by the addition of 0.1 ml NADPH (10 mM) and stopped, after an incubation at 37 °C for 10 min, by the addition of 0.5 ml of an ice-cold aqueous solution of trichloroacetic acid (12.5% *w/v*). Erythromycin *N*-demethylation was estimated determining the produced formaldehyde spectroscopically (412 nm) by the method of Nash [26].

The total hepatic cytochrome P450 content was determined spectroscopically [27], (extinction coefficient 91 $mM^{-1}cm^{-1}$), and the microsomal protein was estimated using bovine serum albumin as a standard [28].

The statistical evaluation of results was performed by the Student's t-test. The lipophicity of guaiazulene was calculated according to the method of Hansch and Leo [29] and calculations of molecular volume were performed by the MacSpartan software (Wavefunction, Inc., USA).

References

☆ Dedicated to Prof. Dr. Dres. h.c. H. Oelschläger on the occasion of his 70th birthday

[1] J. Grogan, M. Shou, E.A. Andrusiak, S. Tamura, J.T.M. Buters, F.J. Gonzalez, K.R. Korzekwa, *Biochem. Pharmacol.* **1995**, *50*, 1509–1515.

[2] P.N. Kourounakis, E. Rekka, *Arch. Pharm.* **1991**, *324*, 161–164.

[3] D.W. Nebert, *Biochem. Pharmacol.* **1994**, *47*, 25–37.

[4] J. George, C. Liddle, M. Murray, K. Byth, G.C. Farrell, *Biochem. Pharmacol.* **1995**, *49*, 873–881.

[5] E.R. Stadtman, *Science* **1992**, *257*, 1220–1224.

[6] J.A. Hinson, N.P. Pumford, D.W. Roberts, *Drug Metab. Rev.* **1995**, *27*, 73–92.

[7] E.A. Rekka, A.P. Kourounakis, P.N. Kourounakis, *Res. Commun. Molec. Pathol. Pharmacol.* **1996**, *92*, 361–364.

[8] W.B. Rathbum, C.E. Killen, A.M. Holleschau, H.T. Nagasawa, *Biochem. Pharmacol.* **1996**, *51*, 1111–1116.

[9] V.E. Kostrubsky, S.G. Wood, M.D. Bush, J. Szakacs, W.J. Bement, P.R. Sinclair, E.H. Jeffery, J.F. Sinclair, *Biochem. Pharmacol.* **1995**, *50*, 1743–1748.

[10] S.D. Nelson, *Drug Metab. Rev.* **1995**, *27*, 147–177.

[11] S. Tuntaterdtum, I.P. Chaudhary, M. Cibull, L.W. Robertson, R.A. Blouin, *Toxicol. Appl. Pharmacol.* **1993**, *123*, 219–225.

[12] E. Rekka, E.O. Ayalogu, D.F.V. Lewis, G.G. Gibson, C. Ioannides, *Arch. Toxicol.* **1994**, *68*, 73–78.

[13] A.N Alexidis, J.N.M. Commandeur, E.A. Rekka, E. Groot, P.N. Kourounakis, N.P.E. Vermeulen, *Env. Toxicol. Pharmacol.* **1996**, *1*, 81–88.

[14] F. J. Gonzalez, *Pharmacol. Ther.* **1990**, *45*, 1–38.

[15] N.P.E. Vermeulen, J.M.C. Bessems, R. Van de Straat, *Drug Metab. Rev.* **1992**, *24*, 367–407.

[16] S. Ohmori, T. Misaizu, T. Nakamura, N. Tanaka, H. Kitagawa, M. Kitada, *Biochem. Pharmacol.* **1993**, *46*, 55–60.

[17] J.R. Halpert, *Ann. Rev. Pharmacol. Toxicol.* **1995**, *35*, 29–53.

[18] D.F.V. Lewis, C. Ioannides, D.V. Parke, *Biochem. Pharmacol.* **1995**, *50*, 619–625.

[19] J. Sangster, *J. Phys. Chem. Ref. Data* **1989**, *18*, 1111–1229.

[20] A.N. Alexidis, E.A. Rekka, P.N. Kourounakis, *Res. Commun. Molec. Pathol. Pharmacol.* **1994**, *85*, 67–72.

[21] F.J. Gonzalez, *Trends Pharmacol. Sci.* **1992**, *13*, 346–352.

[22] I.A. Donatus, Sardjioko, N.P.E. Vermeulen, *Biochem. Pharmacol.* **1990**, *39*, 1869–1875.

[23] E. Rekka, J. Kolstee, H. Timmerman, A. Bast, *Eur. J. Med. Chem.* **1989**, *24*, 43–54.

[24] L.A. Reinke, M.J. Moyer, *Drug Metab. Disp.* **1985**, *13*, 548–552.

[25] S.A. Wrighton, P. Maurel, E.G. Schultz, P.B. Watkins, B. Young, P.S. Guzelian, *Biochemistry* **1985**, *24*, 2171–2178.

[26] T. Nash, *Biochem. J.* **1953**, *55*, 416–421.

[27] T. Omura, R. Sato, *J. Biol. Chem.* **1964**, *239*, 2370–2378.

[28] O.H. Lowry, N.J. Rosenbrough, Y.L. Farr, A.J. Randall, *J. Biol. Chem.* **1951**, *193*, 265–275.

[29] C. Hansch, A. Leo, in *Substituent Constants for Correlation Analysis in Chemistry and Biology*, (Ed.: C. Hansch, A. Leo), Wiley, New York, **1979**.

Received: September 10, 1996 [FP143]