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RIDOGREL: A SELECTIVE INHIBITOR OF THE CYTOCHROME P450-DEPENDENT THROMBOXANE SYNTHESIS*

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Abstract—Ridogrel {(*E*)-5-[[[(3-pyridinyl)[3-(trifluoromethyl)phenyl]methylene]amino]oxy] pentanoic acid} is a potent inhibitor of the P450-dependent human platelet thromboxane A_2 (TxA_2) synthase. Fifty percent inhibition is already achieved at 5.0 ± 0.37 nM. This IC_{50} value is close to half the P450 concentration used, i.e. 10.7 nM. Ridogrel binds to human platelet microsomal P450 as proven by the type II spectral changes induced by the addition of increasing concentrations of ridogrel to solubilized microsomes. The calculated half-maximal spectral change (SC_{50} value) is 3.78 ± 1.79 nM. These results indicate that ridogrel binds stoichiometrically and suggest that inhibition of thromboxane synthesis may originate from liganding of its basic nitrogen to the haem-iron of P450 and from the attachment of the hydrophobic carboxylic side chain to or near the substrate binding place. Ridogrel is a selective inhibitor of the TxA_2 synthase. At a high concentration (10 μM), ridogrel has a slight, if any, effect on the P450-mediated cholesterol synthesis in human liver and hepatoma cells and androgen synthesis from 17α-hydroxy-20-dihydroprogesterone or pregnenolone in subcellular fractions from rat testes. These results indicate that ridogrel is a poor inhibitor of the P450-dependent 14α-demethylase, 17α-hydroxylase and 17,20-lyase. It has, up to 10 μM, no effect on the adrenal mitochondrial 11β-hydroxylase and cholesterol side-chain cleavage enzyme and does not inhibit aromatase activity in human placental microsomes. Ridogrel has no significant effect on the regio- and stereoselective P450-dependent oxidations of testosterone in liver microsomes from unpretreated or from 5-pregnen-3β-ol-20-one-16α-carbonitrile-, phenobarbital- or 3-methylcholanthrene-pretreated male and female Sprague–Dawley rats. It does not interfere with the reduction of testosterone into 5α-dihydrotestosterone and 5α androstane 3β,17β-diol.

Ridogrel (R 68070) is the generic name for (*E*)-5-[[[(3-pyridinyl)[3 - (trifluoromethyl)phenyl]methylene]-amino]oxy]pentanoic acid (Fig. 1). Ridogrel has a dual mechanism of action, namely it inhibits at nanomolar concentrations the platelet microsomal thromboxane A_2 (TxA_2)‡ synthase and blocks at micromolar concentrations the platelet receptors for TxA_2/prostaglandin endoperoxides [1]. In man, a single oral dose of 400 mg ridogrel produces inhibition of platelet TxA_2 synthase, reduces platelet aggregation in platelet-rich plasma induced by the TxA_2 prostaglandin endoperoxide mimic U 46619, collagen or arachidonic acid and prolongs template bleeding times significantly, without affecting plasma coagulation or fibrinolysis [2]. Ridogrel has no effect on the prostacyclin synthase [1].

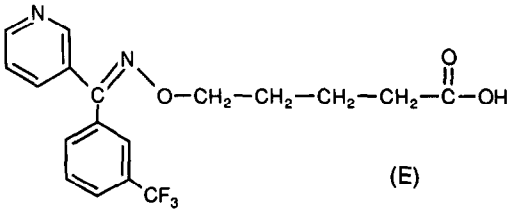

Fig. 1. Chemical structure of ridogrel.

TxA_2 synthase is present in the lung, the kidney, the umbilical cord and blood platelets [3]. The thromboxane synthase from the human platelet 100,000 *g* pellet [4] and the prostacyclin synthase from porcine aortic microsomes [5] have been isolated. These enzymes co-purify with platelet and aortic cytochrome P450, respectively [3–5]. Furthermore, according to their spectral characteristics and catalytic properties these enzymes have been characterized as P450 proteins [3–9].

The present study was designed to examine the interaction of the anti-thrombotic, ridogrel, with the human platelet microsomal P450(s) and thromboxane synthesis, and other microsomal and mitochondrial P450-dependent reactions. The results obtained show that ridogrel has a high affinity for microsomal

* Dedicated to Prof. Dr H. Oelschläger, Frankfurt, on the occasion of his 70th birthday.

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‡ Abbreviations: TxA_2, thromboxane A_2; TxB_2, thromboxane B_2; DTT, dithiothreitol; 3-MC, 3-methylcholanthrene; PCN, 5-pregnen-3β-ol-20-one-16α-carbonitrile; DMSO, dimethyl sulphoxide; DHT, 5α-dihydrotestosterone; PGI_2, prostacyclin; PGH_2, prostaglandin-endoperoxide, 15(*S*)-hydroxy-9,11-[epidioxy]prosta-5,13(*Z*,*E*)-dienoic acid; SC_{50}, half-maximal spectral change; PB, phenobarbital; androstane, 5α-androstane-3β,17β-diol.

P450 from human platelets and is a potent and selective inhibitor of the TxA_2 synthase.

MATERIALS AND METHODS

Preparation of human platelet microsomes. Expired human platelet-rich plasma, obtained from the Belgian Bloodtransfusion Centre, was centrifuged at 20 *g* for 30 min to remove leukocytes and red blood cells. Platelets were collected by repeated centrifugations at 900 *g* for 15 min. After each centrifugation the pellet was resuspended in 50 mM Tris, 10 mM EDTA and 5 mM DTT pH 7.5 and washed three times to remove hemoglobin. The final pellet was resuspended in 10 mM potassium phosphate buffer (pH 7.0) containing 10 mM EDTA, 5 mM glucose, 0.1 mM DTT, 1.15% potassium chloride and 10 mg/L trypsin inhibitor.

Subcellular fractionation and solubilization was done as described by Haurand and Ullrich [4]. Platelets were gassed with nitrogen for 10 min and then sonicated in an ice-water bath with a Branson sonifier (model B30, full power, 3/4″ probe) for 5 × 12 sec with 1 min intervals for cooling. The sonicate was centrifuged for 15 min at 7000 *g* and the supernatant for 60 min at 130,000 *g*. The microsomal pellet was gently resuspended in 10 mM potassium phosphate buffer (pH 7.4) containing 10 mM EDTA, 0.1 mM DTT and 1.15% KCl and centrifuged again for 60 min at 130,000 *g*. The washed microsomes were resuspended, as concentrated as possible, in 10 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v), 1 mM DTT and 1 mM EDTA and stored at −70°. These microsomes were used to determine the TxA_2 synthase activity.

Before solubilization, the microsomal suspension was thawed slowly. Solubilization of the microsomes was achieved by adding, under continuous stirring, a 10 mM potassium phosphate buffer containing 20% glycerol (v/v), 1 mM DTT, 1 mM EDTA, 1.7% (v/v) Lubrol PX and 4.25% (w/v) sodium cholate, to give a final concentration of 0.2% Lubrol PX and 0.5% cholate. After stirring in ice-water for an additional 30 min, the mixture was centrifuged at 130,000 *g* for 60 min. The resulting supernatant was dialysed overnight against 10 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v), 1 mM DTT and 1 mM EDTA. The dialysed supernatant was used for spectrophotometric studies.

Preparation of subcellular fractions from adrenals, testes and placenta. Liver microsomes from control male or female Sprague–Dawley rats (8–9 weeks old) or from rats pretreated for 7 days with PB (1 g/L in the drinking water), or for 3 days with 25 mg/kg body weight 3-MC (i.p. in olive oil) or 50 mg/kg PCN (i.p. in olive oil), were prepared as described by Vanden Bossche *et al.* [10]. Microsomes from human placentas [10], mitochondria from bovine adrenal cortex [11] and S-10,000 fractions (i.e. the supernatant of a 10,000 *g* centrifugation) from Wistar rat testes [12] were prepared as described previously.

Cytochrome P450 isozymes: spectrophotometric analysis. The P450 content was measured as described previously [13].

To trace the spectral transitions of the Soret band of P450 associated with the addition of ridogrel, the microsomal suspension (pH 7.4) containing 0.3 nmol/mL P450 (corresponding to 5.7 mg protein/mL) was divided between the sample and reference cuvettes. A baseline of equal light absorbance was established; increasing concentrations of ridogrel (10 nM–0.5 μM) were added to the sample cuvette and the resulting difference spectra were recorded.

Thromboxane synthase activity. Thromboxane synthesis from [^{14}C]arachidonic acid by human platelet microsomes was studied in a reaction mixture containing in a final volume of 1 mL, 0.1 M potassium phosphate buffer (pH 7.4), 0.2 mg microsomal protein (corresponding to 10.7 nM P450) and 1 μL ridogrel and/or DMSO. After 5 min of preincubation at 37°, 0.25 μCi [^{14}C]arachidonic acid in 50 mM Tris–HCl buffer (pH 7.4) supplemented with 5 mM glucose was added to the reaction mixture. The reaction was stopped after 5 min of incubation at 37° by the addition of 2 mL aceton and 0.1 mL formic acid (10%). Metabolites were extracted twice with 2 mL chloroform and separated by TLC [14]. The amount of radioactive metabolites was determined by liquid scintillation counting. The production of TxA_2 was estimated from the amount of TxB_2 formed [14]. TxB_2 is the stable metabolite of TxA_2.

Cholesterol synthesis. Human Chang liver cells (1×10^6 cells; ATCC CCL 13, Flow Laboratories, Irvine, U.K.) and human hepatoma cells (Hep G_2 cells ATCC HB 8065) were grown at 37° (in a CO_2 incubator) in 2 mL REGA-3 medium supplemented with 5% fetal calf serum. After 24 hr of growth, 1.25 μCi [^{14}C]acetate, ridogrel and/or DMSO were added and cells were grown for another 24 hr. At the end of the incubation period cells were collected by centrifugation, washed with physiological saline and resuspended in 1 mL H_2O and 1 mL 15% KOH in 90% ethanol. The tubes were heated for 2 hr at 85° and the non-saponifiable lipids were extracted with 3/2 volumes of *n*-heptane. Cholesterol and its precursors were separated by TLC on precoated silica gel plates (Merck No. 5554-60F_{254}) and developed in a solvent system [15] consisting of heptane : diisopropylether : acetic acid : ethylacetate (60:40:4:34.7). To visualize the radioactive fractions, the TLC plates were exposed during 3 days to Kodak® autoradiography film. The radioactivity of the different fractions was determined in a liquid scintillation counter.

Cholesterol side-chain cleavage and 11β-hydroxylase. The cortisol synthesis from [^{3}H]11-deoxycortisol (11β-hydroxylase) and pregnenolone sythesis from cholesterol (cholesterol side-chain cleavage) were studied using bovine adrenocortical mitochondria as described by Vanden Bossche *et al.* [10, 11].

Androgen biosynthesis. [^{14}C]Pregnenolone and [^{3}H]17α-hydroxy-20-dihydroprogesterone metabolism by S-10,000 fractions (supernatants of a 10,000 *g* centrifugation, containing the microsomes and the cytosol) of Wistar rat testes was studied as described previously [10, 12].

Aromatase. The effects of ridogrel on the human placental aromatase were studied by measuring the radioactivity of ^3H_2O formed from [1β,2β-^{3}H]-androstenedione [16].

Testosterone metabolism. Liver microsomes from control male or female Sprague–Dawley rats or from

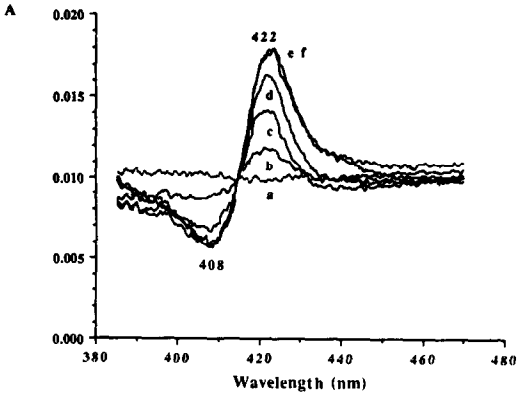

Fig. 2. Optical difference spectra induced by the addition of increasing concentrations (10 nM–0.5 μM) of ridogrel. The sample and reference cuvette contained 0.3 nmol P450/mL. A, absorbance; a, baseline; b, 10 nM; c, 30 nM; d, 0.1 μM; e, 0.2 μM; f, 0.5 μM ridogrel.

rats pretreated with phenobarbital 3-MC or PCN were used to study the effects of ridrogel on the conversion of [4-^{14}C]testosterone into OHT, androstane and hydroxylated metabolites [10]. Testosterone and its metabolites were separated on precoated silicagel 60 F_{254} aluminium plates as described by Waxman *et al.* [17]. Radioactive spots were localized by autoradiography and radioactivity determined.

Protein content. The protein content of the subcellular fractions was determined with the Bio-Rad® protein assay.

RESULTS

Effects on P450s

The P450 content of the detergent-solubilized human platelet microsomes isolated from seven batches is 53.0 ± 11.0 pmol/mg protein. This is similar to the 40.5 pmol/mg protein found by Ullrich and Graf [3]. In the presence of dithionite and carbon monooxide maximum absorption was at 452 nm.

Binding to P450 is shown by the difference spectra induced by the addition of increasing concentrations of ridogrel to solubilized platelet microsomes containing 0.3 μM P450 (Fig. 2). The spectral change is characterized by an absorption peak at 422 nm and a trough (absorption minimum) at about 408 nm. This type II spectral change has been suggested as being due to the formation of a ferrihaemochrome with P450 [18]. As shown in Fig. 2 saturation of the binding spectra was reached between 0.2 and 0.5 μM. When the spectral changes ΔA (422–408 nm) are plotted against the ridogrel concentrations a tritation curve is obtained (Fig. 3) which proceeds almost linearly to the inhibitor concentration that approaches to the P450 concentration (300 nM) used. The spectral dissociation constant K_s, calculated according to Hecker *et al.* [7] from this titration curve, is 0.1 ± 0.051 μM (mean of six experiments ± SD).

Effects on P450-dependent reactions

The affinity of ridogrel for platelet P450(s) was further proven by measuring its effects on the P450-dependent thromboxane synthesis (Fig. 4). Fifty percent inhibition was reached at 5.0 ± 0.37 nM (mean ± SD of seven experiments using microsomes prepared from three batches of platelet-rich plasma).

To measure ridogrel's effects on adrenal, testicular, placental and liver P450s, the following P450-dependent reactions were studied: cholesterol synthesis in Chang and Hep G_2 cells, pregnenolone and cortisol synthesis by bovine adrenal mitochondrial membranes, androgen synthesis by subcellular fractions from rat testes, estrogen synthesis by human placental microsomes and testosterone metabolism by rat liver microsomes (Tables 1 and 2). Ridogrel had up to 10 μM no effect on the 11β-

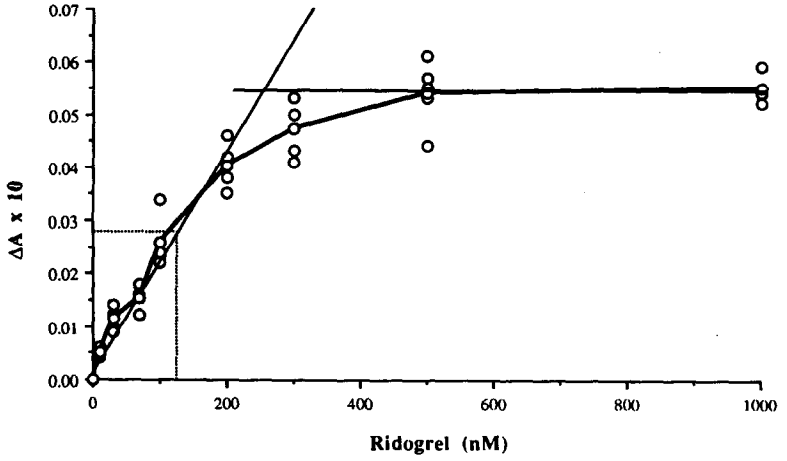

Fig. 3. Titration curve of spectral changes induced by ridogrel. ΔA (422–408 nm) values are taken from type II spectra obtained with platelet microsomes isolated from three batches of human platelet-rich plasma. The full line combines the mean values. The curve fit was obtained with Cricket Graph© software.

Table 1. Effects of ridogrel on P450-dependent reactions

Cells	Membranes	Substrate used	Product(s) formed	% Inhibition at 10 μM ridogrel
Hep G_2	Intact cells	Acetate	Cholesterol	11.3
Chang	Intact cells	Acetate	Cholesterol	5.8
Bovine adrenal	Mitochondria	Cholesterol	Pregnenolone	0.0
		11-Deoxycortisol	Cortisol	0.0
Rat testis	S-10,000*	Pregnenolone	Androgens†	2.2
		17α-Hydroxy-20-dihydro-progesterone	Androgens	14.1
Human placenta	Microsomes	Androstenedione	Estrone‡	0.0

* S-10,000 supernatant of a 10,000 *g* centrifugation.
† Androgens = dehydroepiandrosterone + androstenedione + androstendiol + testosterone.
‡ Measured by ^3H_2O formed from [1β,2β-^{3}H]androstenedione.

hydroxylase and cholesterol side-chain cleavage enzyme and did not affect the aromatase. At this high concentration it inhibited only slightly cholesterol synthesis in human liver and hepatoma cells, and testicular androgen synthesis from pregnenolone or 17α-hydroxy-20-dihydroprogesterone. As shown in Table 2, ridogrel was up to 10 μM a poor inhibitor of the regio- and stereoselective hydroxylations of testosterone and had, in liver microsomal fractions from male and female (8–9 weeks old) Sprague–Dawley rats, no effect on the reduction of testosterone to metabolites of which the R_f value, retention time and mass spectrum resemble that of DHT and androstane (unpublished results).

DISCUSSION

In contrast with other P450 enzymes, both P450s involved in the synthesis of TxA_2 and PGI_2 cannot be reduced by NADPH [5, 9] and no monooxygenase activity is associated with their physiological function [5]. Therefore, the name "haem-thiolate enzyme" instead of P450 enzyme has been proposed [8]. They can also be called P450-dependent isomerases.

The isomerization of PGH_2 to TxA_2 involves endoperoxide activation [19]. From optical and ESR studies on TxA_2 synthase it has been concluded [2, 3] that the ferric enzyme catalyses the cleavage of the endoperoxide bond. Studies of Ullrich and colleagues [3, 4, 19] prove that TxA_2 synthase obtains its specificity through an interaction with the C-9 endoperoxide oxygen: PGH_2 is bound to the active site of TxA_2 synthase with its C-9 oxygen at the haem iron. Homolytic scission of the endoperoxide bond gives rise to the alkoxy radical which undergoes Fe(IV)-oxidation to the carbocation, followed by ionic rearrangement to TxA_2 [20].

The isomerization of PGH_2 to PGI_2 also involves

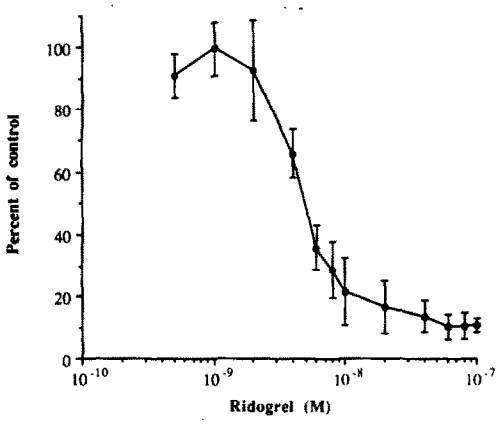

Fig. 4. Inhibition of the synthesis of TxB_2 (the stable metabolite of TxA_2) from [^{14}C]arachidonic acid by non-solubilized human platelet microsomes prepared from three different batches. After incubation the metabolites formed were extracted and separated by TLC. Under control conditions 15.9% of the radioactivity found in the extract was recovered in a compound with the same R_f value as TxB_2. Results (mean values ± SD of seven experiments) are expressed as percentages of control. Ridogrel concentrations used: 0.5 nM–0.1 μM.

Table 2. Effects of ridogrel on testosterone metabolism

Testosterone metabolites	% Inhibition at 10 μM UT M	UT F	PB M	PB F	3-MC M	3-MC F	PCN M	PCN F
2α-OH-	0	—†	2	22	0	28	0	11
6α-OH-	40	—†	31	15	9	45	32	25
6β-OH-	28	—†	3	24	10	25	0	0
7α-OH-	0	—†	0	47	0	23	0	48
16α-OH-	0	29	10	5	9	16	27	16
16β-OH-	33	—†	0	30	0	47	9	27
Androstane	3	9	5	0	16	0	12	6
DHT	0	0	0	0	0	0	0	0

The incorporation of radioactivity from [4-^{14}C]testosterone into hydroxylated (OH) metabolites and DHT and androstane by liver microsomes from male (M) and female (F) rats in the presence of increasing concentrations (0.1–10 μM) of ridogrel. The % inhibitions in the presence of 10 μM only are given. Liver microsomes were prepared from untreated (UT) and from PB-, 3-MC- or PCN-pretreated rats. Controls are incubated in the presence of DMSO. Steroids are separated by TLC and identified by comparing the R_f values with those of standards and/or by gas chromatographic–mass spectrometric analysis [13].

* Incorporation of radioactivity into the steroids formed under control conditions was <1000 dpm.

endoperoxide activation. The P450-dependent PGI_2 synthase obtains its specificity through an interaction with the endoperoxide oxygen at C-11 [19, 20]. This difference between the TxA_2 and the PGI_2 synthases may be at the origin of the fact that ridogrel has no effect on the prostacyclin synthase [1] whereas, as shown in this paper, it has a high affinity for platelet P450 and is a potent inhibitor of the TxA_2 synthase. Fifty percent inhibition is achieved at about 5 pmol/mL, an IC_{50} value which is close to half the P450 content used (i.e. 10.7 pmol/mL).

This inhibitory effect might originate from ridogrel's affinity for platelet P450. Indeed, the type II spectral change observed when ridogrel is added to microsomal suspensions suggests that it coordinates by its pyridine nitrogen to the haem iron, i.e. at the catalytic site of the enzyme. Studies of Hecker *et al.* [7] show that the pyridine derivative, OKY-1581, binds stoichiometrically to the solubilized TxA_2 enzyme and that the IC_{50} value is close to the calculated spectral half-titration value. The K_s value (0.11 ± 0.051 μM) found with ridogrel is much higher than the IC_{50} value. However, the IC_{50} value is obtained with a 28 times lower P450 content. When the SC_{50} values are calculated with the P450 content used for the TxA_2 synthase experiment a SC_{50} value of 3.78 ± 1.79 nM is found. Thus, the experimental IC_{50} value is also close to the calculated spectral half-titration value. This suggests that ridogrel also binds stoichiometrically. The fact that the IC_{50} value and SC_{50} value are almost identical suggests that the substrate PGH_2 is unable to compete with the inhibitor [7]. This may originate from the fact that thromboxane synthase has a rather low affinity for the endoperoxide ($K_m = \pm 20$ μM [7]).

The differences found between the P450-dependent isomerases and the P450s with monooxygenase activity (*supra vide*) suggest that the sensitivity of the latter to ridogrel may differ from that of the P450 involved in the sythesis of thromboxane A_2. Indeed, ridogrel does not affect P450-dependent cholesterol ($P450_{LI}$), pregnenolone ($P450_{XIA1}$), androgen ($P450_{XVIIA1}$) and estrogen ($P450_{XIXA1}$) synthesis. It has no effect on the 11β-hydroxylase ($P450_{XIB1}$) and has a slight effect on the regio- and stereoselective hydroxylations of testosterone by liver microsomal P450s from unpretreated rats (e.g. $P450_{IIC11}$) and by P450 forms expressed in rats in response to chemical stimuli i.e. PB ($P450_{IIB1}$, $P450_{IIB2}$), 3-MC ($P450_{IA1}$), and PCN ($P450_{IIIA1}$, $P450_{IIIA2}$). It also does not inhibit the reduction of testosterone to DHT and androstane.

The preceding results and discussion demonstrate that ridogrel is a potent and highly selective inhibitor of P450-dependent thromboxane synthesis.

REFERENCES

1. De Clerck F, Beetens J, de Chaffoy de Courcelles D, Freyne E and Janssen PAJ, R 68070: thromboxane A2 synthetase inhibition and thromboxane A2/prostaglandin endoperoxide receptor blockade combined in one molecule-I. Biochemical profile *in vitro*. *Thromb Haemost* **61**: 35–42, 1989.
2. De Clerck F, Beetens J, Van de Water A, Vercammen E and Janssen PAJ, R 68070: thromboxane A2 synthetase inhibition and thromboxane A2/prostaglandin endoperoxide receptor blockade combined in one molecule-II. Pharmacological effects *in vivo* and *ex vivo*. *Thromb Haemost* **61**: 43–49, 1989.
3. Ullrich V and Graf H, Prostacyclin and thromboxane synthase as P-450 enzymes. *TIPS* **5**: 352–355, 1984.
4. Haurand M and Ullrich V, Isolation and characterization of thromboxane synthase from human platelets as a cytochrome P-450 enzyme. *J Biol Chem* **260**: 15059–15067, 1985.
5. Graf H and Ullrich V, Prostacyclin synthase as a cytochrome P450 enzyme. In: *Cytochromes P-450, Biochemistry, Biophysics and Environmental Implications* (Eds. Hietanen E, Laitinen M and Hänninen O), pp. 103–106. Elsevier Biomedical Press, Amsterdam, 1982.
6. Ullrich V and Haurand M, Thromboxane synthase as a cytochrome P450 enzyme. In: *Advances in Prostaglandin, Thromboxane and Leukotriene Research* (Eds. Samuelson B, Paoletti R and Ramwell P), pp. 105–110. Raven Press, New York, 1983.
7. Hecker M, Haurand M and Ullrich V, Spectral studies on structure–activity relationships of thromboxane synthase inhibitors. *Eur J Biochem* **157**: 217–223, 1986.
8. Hecker M, Baader WJ, Weber P and Ullrich V, Thromboxane synthase catalyses hydroxylations of prostaglandin H2 analogs in the presence of iodosylbenzene. *Eur J Biochem* **169**: 563–569, 1987.
9. Ullrich V, Castle L and Weber P, Spectral evidence for the cytochrome P450 nature of prostacyclin synthetase. *Biochem Pharmacol* **30**: 2033–2036, 1981.
10. Vanden Bossche H, Willemsens G, Roels I, Bellens D, Moereels H, Coene M-C, Le Jeune L, Lauwers W and Janssen PAJ, R 76713 and enantiomers: selective, nonsteroidal inhibitors of the cytochrome P450-dependent oestrogen synthesis. *Biochem Pharmacol* **40**: 1707–1718, 1990.
11. Vanden Bossche H, Willemsens G, Cools W and Bellens D, Effects of etomidate on steroid biosynthesis in subcellular fractions of bovine adrenals. *Biochem Pharmacol* **33**: 3861–3868, 1984.
12. Vanden Bossche H, Lauwers W, Willemsens G and Cools W, The cytochrome P450-dependent C17,20-lyase in subcellular fractions of the rat testis. In: *Microsomes and Drug Oxidations* (Eds. Boobis AR, Caldwell J, de Matteis F and Elcombe CR), pp. 63–73. Taylor and Francis, London, 1985.
13. Vanden Bossche H, Bellens D, Cools W, Gorrens J, Marichal P, Verhoeven H, Willemsens G, De Coster R, Beerens D, Haelterman C, Coene M-C, Lauwers W and Le Jeune L, Cytochrome P-450: target for itraconazole. *Drug Dev Res* **8**: 287–298, 1986.
14. De Clerck F and Van Nueten JM, Platelet-mediated vascular contractions. Inhibition by flunarizine, a calcium-entry blocker. *Biochem Pharmacol* **32**: 765–771, 1983.
15. Vanden Bossche H, Willemsens G, Marichal P, Cools W and Lauwers W, The molecular basis for the antifungal activity of *N*-substituted azole derivatives. Focus on R 51211. In: *Mode of Action of Antifungal Agents* (Eds. Trinci APJ and Ryley JF), pp. 321–341. Cambridge University Press, Cambridge, 1984.
16. Vanden Bossche H, Marichal P, Willemsens G, Bellens D, Gorrens J, Roels I, Coene M-C, Le Jeune L and Janssen PAJ, Saperconazole: a selective inhibitor of the cytochrome P-450-dependent ergosterol synthesis in *Candida albicans, Aspergillus fumigatus* and *Trichophyton mentagrophytes*. *Mycoses* **33**: 335–352, 1990.
17. Waxman DJ, Ko A and Walch C, Regioselectivity and stereoselectivity of androgen hydroxylations catalysed by cytochrome P-450 isozymes purified from pheno-

barbital-induced rat liver. *J Biol Chem* **258**: 11937–11947, 1983.
18. Schenkman JB, Sligar SG and Cinti DL, Substrate interaction with cytochrome P-450. In: *Hepatic Cytochrome P-450 Monooxygenase System* (Eds. Schenkman JB and Kupfer D), pp. 587–615. Pergamon Press, Oxford, 1982.
19. Hecker M and Ullrich V, On the mechanism of prostacyclin and thromboxane A2 biosynthesis. *J Biol Chem* **264**: 441–450, 1989.
20. Ullrich V, Haurand M and Hecker M, The significance of the heme-thiolate structure of prostacyclin and thromboxane synthase. In: *Cytochrome P-450: Biochemistry and Biophysics* (Ed. Schuster I), pp. 41–48. Taylor and Francis, London, 1989.