ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
16 (1997) 223–229

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Relationship between lipophilicity and binding to human serum albumin of arylpropionic acid non-steroidal anti-inflammatory drugs

Laurence Deschamps-Labat [a], Fabienne Péhourcq [a], Maryline Jagou [a], Bernard Bannwarth [b,*]

[a] *Department of Pharmacology, EA 525, Groupe hospitalier Pellegrin, Université de Bordeaux II, 33076 Bordeaux Cedex, France*
[b] *Departments of Rheumatology and Therapeutics, EA 525, Groupe hospitalier Pellegrin, Université de Bordeaux II, 33076 Bordeaux Cedex, France*

Received 17 September 1996; received in revised form 2 January 1997

Abstract

A possible relationship between lipophilicity and binding to human serum albumin was investigated for 11 arylpropionate non-steroidal anti-inflammatory drugs. The lipophilic parameter was determined by a reversed-phase high-performance liquid chromatographic procedure as the capacity factor (k'). The binding of arylpropionic acids to human serum albumin was studied in vitro by equilibrium dialysis. For each compound, a Scatchard analysis was performed considering two classes of binding sites characterized by high- and low-affinity constants, K_1 and K_2, respectively. A linear relationship was found between lipophilicity and binding parameters, n_1K_1 ($r = 0.88$, $P < 0.0005$) and n_2K_2 ($r = 0.96$, $P < 0.0002$). These results suggest the role of hydrophobic interactions in the binding of arylpropionic acids to human serum albumin. © 1997 Elsevier Science B.V.

Keywords: Arylpropionic acids; Lipophilicity; Non-steroidal anti-inflammatory drugs; Protein binding; Quantitative structure–activity relationship

1. Introduction

Arylpropionic acid derivatives represent the main group of available non-steroidal anti-inflammatory drugs (NSAIDs). Furthermore, they constitute a homogeneous chemical group with a common chiral acid centre (Table 1). These drugs are currently marketed as 50/50 racemic mixtures with the exception of *S*-naproxen [1]. Their plasma protein binding is generally over 99% [2]. The existence of two specific NSAID binding sites on human serum albumin (HSA) has been described and almost all NSAIDs appear to interact mainly with site II, also called the benzodiazepine site, and secondarily with site I, also called the warfarin site [3–5].

Few experiments investigated the relationships between the physicochemical parameters of drugs

* Corresponding author. Tel.: + 33 557571560.

PII S0731-7085(97)00017-4

and their protein binding characteristics. Lipophilicity appears as one of the most important factors involved in the extent of drug binding [6,7]. Accordingly, we studied such a relationship in a series of 11 arylpropionic acid NSAIDs.

Several chromatographic approaches have been proposed to avoid the difficulties in performing log P measurements by the classic 'shake-flask' method [8,9]. Thus, the hydrophobic parameter was measured as the logarithmic chromatographic capacity factor values ($\log k'_w$) by reversed-phase high-performance liquid chromatography (RP-HPLC). The binding of arylpropionic acids to human serum albumin (HSA) was studied in vitro by equilibrium dialysis. For each compound, a Scatchard analysis was performed, allowing the determination of the number of binding sites (n) and the association constants (K).

2. Materials and methods

2.1. Chemicals

Flurbiprofen and ibuprofen (Boots, Nottingham, UK), pirprofen (Ciba-Geigy, Rueil-Malmaison, France), tiaprofenic acid (Roussel-Uclaf, Romainville, France), alminoprofen (E. Bouchara, Levallois, France) were generously supplied. Carprofen, fenbufen, fenoprofen, indoprofen, ketoprofen, suprofen and essentially fatty-acid-free HSA were obtained from Sigma, St Louis, USA.

All chemicals and solvents were of analytical reagent or HPLC grade. Water was deionized and doubly-glass distilled.

2.2. Apparatus and chromatographic conditions

The high-performance liquid chromatography procedures were performed using a Waters Assoc. apparatus equipped with a WISP 710B model automatic injector, a Model M45 pump and a Lambda-Max Model Ultraviolet detector. The compounds were chromatographed on an Ultrabase C18 column (Shandon, 5 μm particle size, 250 × 4.6 mm i.d) at a flow rate of 2 ml min^{-1}. They were detected at adequate wavelengths in the range 230–290 nm. The various mobile phase compositions ranged from 30 to 70% methanol with 0.06 M phosphate buffer (pH 7.4) (v/v). The detector output was recorded on a Data Jet integrator (Spectra Physics).

2.3. Determination of $\log k'_w$

All stock solutions contained 1 mg ml^{-1} of each drug. They were prepared in methanol and subsequently diluted with water to the final injected concentrations (50 μl at 50 μg ml^{-1}). According to their chromatographic behaviour, the retention time (t_r) of each arylpropionic acid was determined in triplicate at six different methanol–phosphate buffer mobile phase mixtures. At each mobile phase composition at pH 7.4, the capacity factor was calculated through the formula:

$$k' = \frac{t_r - t_0}{t_0}$$

where t_0 is the column dead-time of the system and was measured as the time from the injection to the first distortion of the baseline after drug injection. The $\log k'$ values at 100% aqueous mobile phase ($\log k'_w$) were obtained from the y-intercept of plots $\log k'$ versus percent of methanol in the eluent [10].

2.4. Protein binding

The protein binding was studied in vitro by equilibrium dialysis [11]. A rotative Dianorm apparatus (Braun Science Tec, Les Ulis, France) was equipped with 2-ml macrocells. The two compartments of these cells were separated by a Diachema membrane (cut-off 5000 Da). HSA solutions (600 μl) were freshly prepared in isotonic phosphate buffer (pH 7.4) and immediately dialysed against an equal volume of this buffer at 37°C for 5 h. The concentration of albumin was equal to 40 g l^{-1} in every case, and the total arylpropionic acid concentrations were between 60 and 1000 μg ml^{-1} in phosphate buffer compartment. Dialysis was performed in triplicate for each drug. The absence of NSAID adsorption on the membrane or cell surface was verified by carrying out an experiment without protein. In each dialysis compartment, the drug concentrations were measured by a previously described HPLC method [12]. Briefly, the acidified samples were extracted by diethylether.

Table 1
Chemical structure of arylpropionic acid derivatives studied

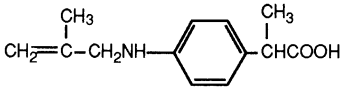
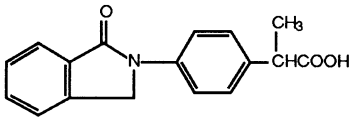
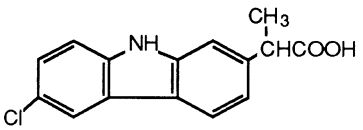
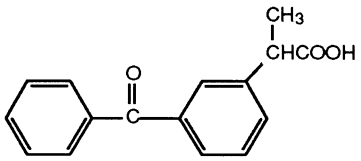
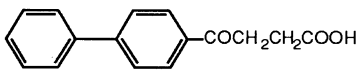
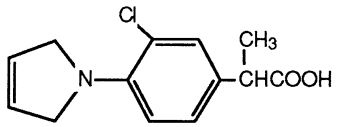
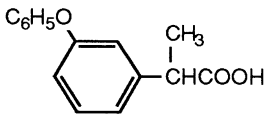
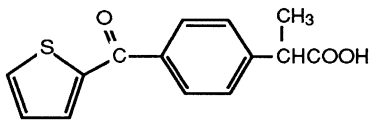
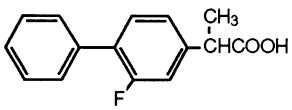
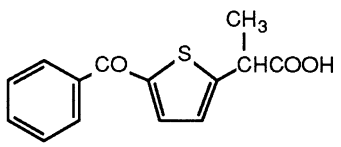
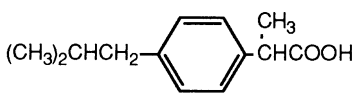
Compound	Chemical structure	Compound	Chemical structure
Alminoprofen		Indoprofen	
Carprofen		Ketoprofen	
Fenbufen		Pirprofen	
Fenoprofen		Suprofen	
Flurbiprofen		Tiaprofenic acid	
Ibuprofen			

Table 2
Log k'_w values and binding parameters of arylpropionic acid NSAIDs

Compounds	log k'_w	n_1 (mean ± S.D.)	K_1 (mean ± S.D.)	n_2 (mean ± S.D.)	K_2 (mean ± S.D.)
Alminoprofen	2.87	0.55 ± 0.03	706.63 ± 130.15	3.64 ± 0.27	3.32 ± 0.24
Carprofen	3.85	1.92 ± 0.03	1185.71 ± 193.24	3.93 ± 0.15	59.47 ± 9.75
Fenbufen	3.32	1.48 ± 0.19	541.75 ± 87.41	10.23 ± 0.86	8.07 ± 1.35
Fenoprofen	3.35	1.01 ± 0.12	1017.63 ± 205.27	3.89 ± 0.37	18.34 ± 3.45
Flurbiprofen	3.59	0.97 ± 0.09	862.35 ± 25.17	5.53 ± 0.11	18.91 ± 3.33
Ibuprofen	2.99	0.98 ± 0.14	434.56 ± 65.56	5.12 ± 0.04	8.59 ± 0.89
Indoprofen	2.62	0.56 ± 0.08	172.90 ± 42.62	4.13 ± 0.17	4.15 ± 1.12
Ketoprofen	3.20	1.88 ± 0.15	492.37 ± 28.15	6.59 ± 0.38	4.99 ± 1.33
Pirprofen	3.05	1.41 ± 0.20	825.10 ± 129.56	4.87 ± 0.45	8.12 ± 1.24
Suprofen	2.57	1.09 ± 0.07	244.16 ± 61.37	5.59 ± 0.82	1.52 ± 0.16
Tiaprofenic acid	2.61	1.58 ± 0.12	131.80 ± 23.99	4.76 ± 0.49	2.24 ± 0.52

n, Number of binding sites per mole of albumin for site I (n_1) and site II (n_2); K, affinity constant (mM^{-1}) for site I (K_1) and site II (K_2).

According to the chromatographic behaviour of each arylpropionic acid, various internal standards as well as different mobile phase compositions of acetonitrile–0.06 M phosphate buffer at pH 7.4 (v/v) were used.

2.5. Data analysis

The results of each dialysis experiment were analyzed according to Scatchard's equation, assuming two independent classes of binding sites:

$$r = \frac{n_1 K_1 F}{1 + K_1 F} + \frac{n_2 K_2 F}{1 + K_2 F}$$

where n represents the number of binding sites per mole of albumin for site I (n_1) and site II (n_2), K the association constant for site I (K_1) and site II (K_2), and F the molar concentration of free drug. Accordingly, r is the number of moles of drug bound per mole of protein [13,14]. The binding parameters were estimated by a least square regression analysis program (MicroPharm®) using a digital computer.

3. Results

3.1. Lipophilicity of arylpropionic acids

The lipophilic parameters were evaluated in a reversed-phase chromatographic system. The log k'_w values ranged from 2.57 (suprofen) to 3.85 (carprofen) (Table 2).

3.2. Binding of arylpropionic acids to HSA

According to Scatchard's equation, two successive saturable processes were observed (Fig. 1). The number of primary and secondary binding sites, n_1 and n_2, and the affinity constants, K_1 and K_2, are listed in Table 2. The number of high-affinity binding sites per mole of HSA (n_1) was between 0.55 (alminoprofen) and 1.92 (carprofen).

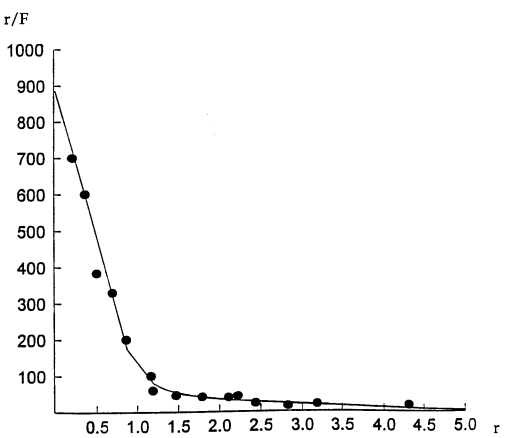


Fig. 1. Scatchard plot of data obtained from equilibrium dialysis on ketoprofen–HSA binding: F, molar concentration of free drug; r, number of moles of drug bound per mole of protein.

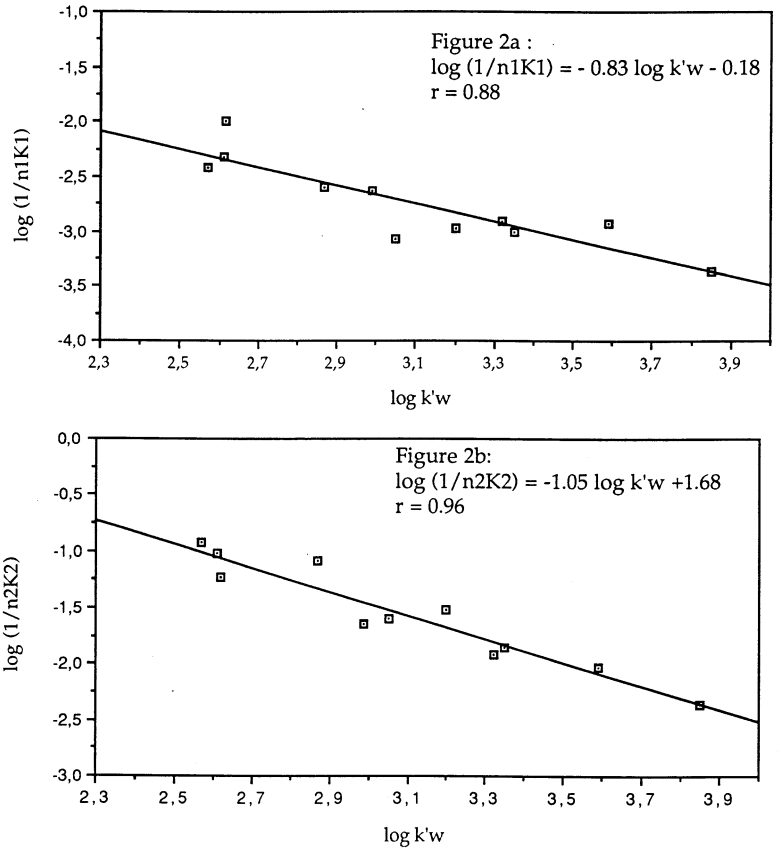


Fig. 2. Plots of (a) $\log(1/n_1K_1)$ vs. $\log k'_w$ and (b) $\log(1/n_2K_2)$ vs. $\log k'_w$.

The number of low-affinity binding sites (n_2) was higher, ranging from 3.64 (alminoprofen) to 10.23 (fenbufen).

3.3. Relationship between binding parameters and lipophilicity for quantitative structure–activity relationship (QSAR) study

Linear relationships between lipophilicity and binding parameters were observed. Significant linear regressions were found between $\log k'_w$ and $\log(1/n_1K_1)$ (Fig. 2(a)) or $\log(1/n_2K_2)$ values (Fig. 2(b)), respectively. For primary sites, the relationship was given by the following equation:

$$\log\left(\frac{1}{n_1K_1}\right) = -0.83 \log k'_w - 0.18$$

where $n = 11$, $r = 0.88$, and $F = 30.91$ ($P < 0.0005$).

For the second class of sites, the relationship between $\log k'_w$ and binding parameters resulted in the following regression equation:

$$\log\left(\frac{1}{n_2K_2}\right) = -1.05 \log k'_w + 1.68$$

where $n = 11$, $r = 0.96$, and $F = 95.54$ ($P < 0.0002$).

Suprofen exhibited the lowest lipophilicity ($\log k'_w = 2.57$) and presented a low affinity to HSA for both types of sites. By contrast, carprofen was a more lipophilic compound ($\log k'_w = 3.85$) with higher affinity.

Table 3
Binding characteristics of arylpropionic acid NSAIDs

	n_1	K_1 (mM^{-1})	n_2	K_2 (mM^{-1})	Experimental conditions	References
Carprofen	1.2	3700	4.0	130	HSA, 3.5 g l^{-1}; $T = 25°C$; $t = 13$ h	[5,26,27]
Ibuprofen	0.8	2730	6.27	19.5	HSA, 10 g l^{-1}; $T = 37°C$; $t = 36$ h	[20]
	1.1	2500	4.2	150	HSA, 3.5 g l^{-1}; $T = 25°C$; $t = 13$ h	[5,26,27]
Ketoprofen	1.40	620	4.37	7.3	HSA, 40 g l^{-1}; $T = 37°C$; $t = 3$ h	[21]
Pirprofen	0.9	390	2.9	8	HSA, 1 g l^{-1}; $T = 37°C$; $t = 12$ h	[25]
Suprofen	1.05	140	2.9	376	HSA, ND; $T = 25°C$; $t = 6$ h	[28]

n, Number of binding sites per mole of albumin for site I (n_1) and site II (n_2); K, affinity constant (mM^{-1}) for site I (K_1) and site II (K_2); T, dialysis temperature; t, dialysis time; HSA, human serum albumin; ND, not defined.

4. Discussion

To obtain further insights into the binding of arylpropionic acid NSAIDs, we investigated a possible relationship between the lipophilicity and the binding properties of a series of 11 compounds. As already described, the lipophilicity seems to be involved in the extent of drug binding to HSA [7,15,16].

In the present study, the lipophilic character of arylpropionic acids was measured as the retention factor k' by RP-HPLC. A good correlation was generally found between this capacity factor and the classic partition coefficient P measured in an n-octanol system. As a result, the log k'_w values can be used in QSAR studies [17,18]. The Scatchard analysis of equilibrium dialysis data allowed two types of binding sites to be characterized for arylpropionic acids. The first binding site, also called the benzodiazepine site, is characterized by a value of n less than 2 and a high affinity constant. Conversely, the second class or warfarin site exhibits a high site number and a low affinity constant. The number of the second class binding site appears to be relatively high for some drugs including fenbufen, flurbiprofen, ibuprofen, ketoprofen and suprofen. Interestingly, competition studies using site I and site II markers as displacing agents suggest that some arylpropionic acids like suprofen may bind to other minor sites on HSA [19].

Since equilibrium dialysis assays were performed under various experimental conditions, it is not surprising that discrepant results have been reported in the literature (Table 3). In fact, the number of binding sites (n) and the affinity constant (K) depend on the albumin concentration, the dialysis temperature and the dialysis time [20,21].

Our study was conducted under experimental conditions close to physiological ones with the dialysis temperature at 37°C and HSA concentration at 40 g l^{-1}. Thus, it would be useful to define standardized conditions for further experiments.

Scatchard's equation suggests that drug binding to HSA is directly dependent on the product of specific binding parameters nK (see Section 2). To obtain the most expressive description of the binding parameters for correlations with lipophilicity, the total affinities n_1K_1 and n_2K_2 were thus calculated [22]. Accordingly, we found a significant linear relationship between the products nK and the lipophilicity log k'_w.

This relationship suggests that hydrophobic forces are involved in drug binding to HSA. However, the binding phenomenon cannot be explained on the basis of a single intermolecular force model [23,24]. Drugs of acidic character, such as arylpropionic acids, are almost completely ionized at physiological pH, suggesting the role of other forces such as electrostatic ones. This hypothesis is strengthened by thermodynamic studies which showed the electrostatic nature of the interactions between albumin and drugs [25]. Rahman et al. [26,27] described Van der Waals interactions, as a consequence of hydrophobic interactions in the binding of carprofen to HSA. The carboxyl group belonging to the propanoate portion of carprofen was directly involved in the enthalpic value, and hence in the high-affinity

binding of this drug. This is in agreement with the findings reported by Maruyama et al. [28], who demonstrated that the carboxyl group was crucial for the binding of suprofen to HSA.

Conformational factors may play a role in the binding of NSAIDs to HSA [29]. Various results on the stereoselective binding of chiral drugs to plasma proteins have also been reported [1]. Arylpropionic acids are characterized by a chiral carbon in the α-position to the carboxyl group, and have two enantiomeric forms, *S* and *R*. In spite of a common lipophilic partition coefficient, differences between the *R*- and *S*-enantiomers affinity constants for the primary and secondary sites of albumin may be observed. Dubois et al. [21] reported that the binding constants of ketoprofen enantiomers depend on whether these isomers are studied alone or as a racemic mixture. Hence, the presence of one enantiomer affects the protein binding of the antipode. In fact, the relationship between lipophilicity and HSA binding did not apply to naproxen (data not shown). The latter is marketed as a pure *S*-enantiomer, in contrast to the other arylpropionic acid derivatives, which are available as 50/50 racemic mixtures.

5. Conclusion

Overall, binding of arylpropionic acids to HSA increased linearly with lipophilicity. This feature is restricted to the experimental conditions used in our study, and to the NSAIDs with lipophilic parameter $\log k'_w$ between 2.57 (suprofen) and 3.85 (carprofen). Under other experimental conditions, some factors including electrostatic and conformational forces may play an increasing role, so that the linear relationship between lipophilicity and total affinity to HSA may become inappropriate.

Acknowledgements

The authors thank E. Deridet and B. Martinez for their skilled technical assistance.

References

[1] F. Lapicque, N. Muller, E. Payan and N. Dubois, Clin. Pharmacokinet., 25 (1993) 115–125.
[2] J. Lin, D. Cochetto and D. Duggan, Clin. Pharmacokinet., 12 (1987) 402–432.
[3] K. Fehske, W. Müller and U. Wollert, Biochem. Pharmacol., 30 (1981) 687–692.
[4] J.P. Tillement, G. Houin, R. Zini, S. Urien, E. Albengres, J. Barré, M. Lecomte, P. D'Athis and B. Sebille, Adv. Drug Res., 13 (1984) 59–94.
[5] M. Rahman, K. Yamasaki, Y. Shin, C. Lin and M. Otagiri, Biol. Pharm. Bull., 16 (1993) 1169–1174.
[6] M. Làznicek and A. Làznickovà, J. Pharm. Biomed. Anal., 13 (1995) 823–828.
[7] F. Péhourcq, A. Radouane, L. Labat and B. Bannwarth, Pharm. Res., 12 (1995) 1535–1538.
[8] C. Altomare, S. Cellamare, A. Carotti and M. Ferappi, Il Farmaco, 49 (1994) 393–401.
[9] J. Sabatka, D. Minick, T. Shumaker, G. Hodgson and D. Brent, J. Chromatogr., 384 (1987) 349–356.
[10] F. Demotes-Mainard, J. Thomas, J.J. Bosc, G. Devaux and C. Jarry, J. Liq. Chromatogr., 16 (1993) 767–776.
[11] H. Kurz and B. Fichtl, Drug Metab. Rev., 14 (1983) 467–510.
[12] F. Péhourcq, F. Lagrange, L. Labat and B. Bannwarth, J. Liq. Chromatogr., 18 (1995) 3969–3979.
[13] J. Vallner, J. Perrin and S. Wold, J. Pharm. Sci., 65 (1976) 1182–1187.
[14] K. Giacomini, A. Abang and T. Blaschke, Br. J. Clin. Pharmacol., 14 (1982) 752–754.
[15] J. Seydel and K. Schaper, Pharmacol. Ther., 15 (1982) 131–182.
[16] G. Wilkinson, Drug Metab. Rev., 14 (1983) 427–465.
[17] R. Kaliszan, J. Chromatogr., 220 (1981) 71–83.
[18] K. Valko, J. Liq. Chromatogr., 10 (1987) 1663–1686.
[19] S. Rahim and A.F Aubry, J. Pharm. Sci., 84 (1995) 949–952.
[20] J. Whitlam, M. Crooks, K. Brown and P. Padersen, Biochem. Pharmacol., 28 (1978) 675–678.
[21] N. Dubois, F. Lapicque, M. Abiteboul and P. Netter, Chirality, 5 (1993) 126–134.
[22] J. Oravcova, V. Mlynarik, S. Bystricky, L. Soltes, P. Szalay, L. Bohacik and T. Trnovec, Chirality, 3 (1991) 412–417.
[23] G. Sudlow, D. Birkett and D. Wade, Mol. Pharmacol., 11 (1975) 824–832.
[24] G. Sudlow, D. Birkett and D. Wade, Mol. Pharmacol., 12 (1976) 1052–1061.
[25] M. Otagiri, K. Masuda, T. Imai, Y. Imamura and M. Yamasaki, Biochem. Pharmacol., 38 (1989) 1–7.
[26] M. Rahman, T. Maruyama, T. Okada, K. Yamasaki and M. Otagiri, Biochem. Pharmacol., 46 (1993) 1721–1731.
[27] M. Rahman, T. Maruyama, T. Okada, T. Imai and M. Otagiri, Biochem. Pharmacol., 46 (1993) 1733–1740.
[28] T. Maruyama, C. Lin, K. Yamasaki, T. Miyoshi, T. Imai, M. Yamasaki and M. Otagiri, Biochem. Pharmacol., 45 (1993) 1017–1026.
[29] U. Kragh-Hansen, Pharmacol. Rev., 33 (1981) 17–53.