Nonsteriodal Antiinflammatory Agents, XVII[1]:

Inhibition of Bovine Cyclooxygenase and 5-Lipoxygenase by *N*-Alkyldiphenyl-pyrrolyl Acetic and Propionic Acid Derivatives[+]

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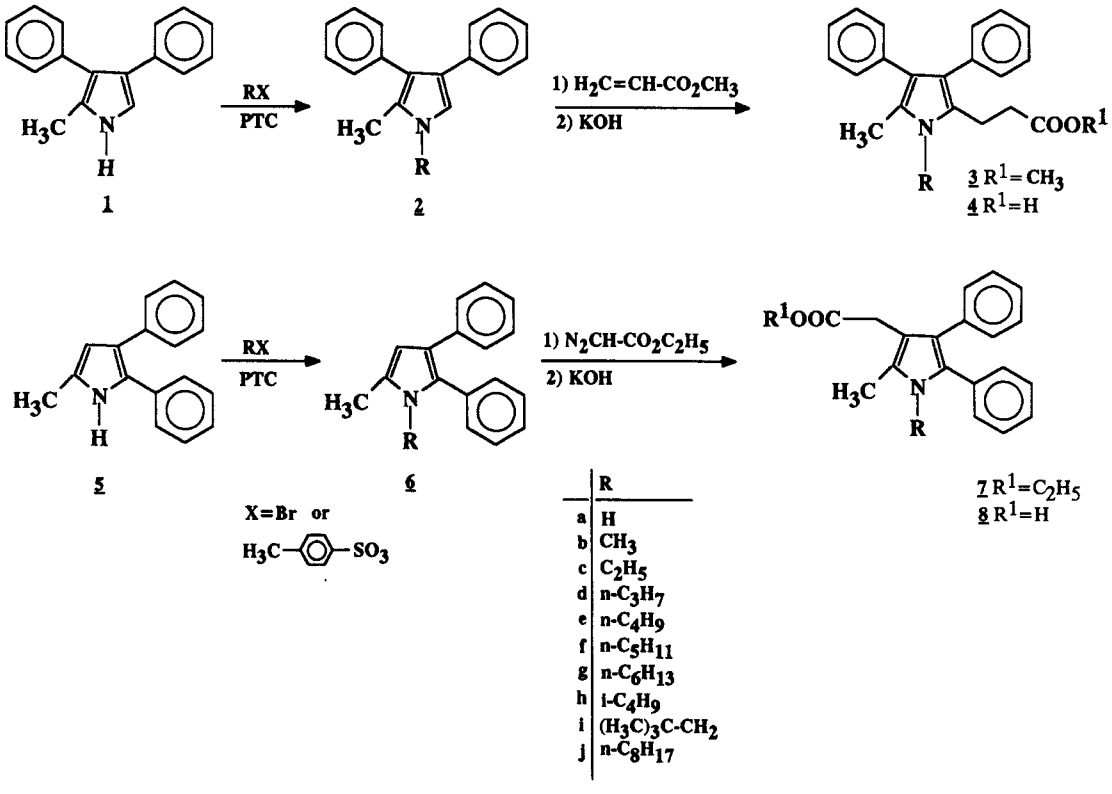
A series of 19 *N*-alkyl-diarylpyrrolyl-acetic and -propionic acid derivatives was synthesized and tested. Using bovine blood as enzyme source the inhibition of cyclooxygenase and 5-lipoxygenase, respectively, was applied to determine the antiinflammatory activity. In general all compounds tested inhibit 5-lipoxygenase more effectively than cyclooxygenase. A structure-activity relationship is discussed.

Nichtsteroidale Antiphlogistica, 17. Mitt.: Inhibierung von Rinder-Cyclooxygenase und 5-Lipoxygenase durch *N*-Alkyldiphenylpyrrolyl-Essigsäure- und Propionsäure-Derivate

Die Synthese und inhibitorische Aktivität von 19 *N*-Alkylphenyl-substituierten Pyrrolyl-essigsäure- bzw. -propionsäure-Derivaten wird beschrieben. Als Testsysteme dienen die aus Rinderblut gewonnenen Enzyme Cyclooxygenase und 5-Lipoxygenase. Die Hemmung beider Enzyme, mit einer stärkeren Beeinflussung der 5-Lipoxygenase, wird unter Einbeziehung struktureller Variationen diskutiert.

Introduction

Nonsteroidal antiinflammatory drugs (NSAID) are widely used agents but the current therapy for chronic inflammatory diseases is still unsatisfactory. The aryl and heteroaryl acetic and propionic acids - for *e.g.* tolmetin (1-methyl-5-*p*-toluoyl-pyrrol-2-yl)acetic acid) is introduced in the therapy of rheumatoid diseases - are highly developed and investigated over the past decades[2]. Previous experimental and pharmacological studies in the field of diaryl dihydropyrrolizinyl acids[3] have indicated an antiinflammatory activity, making the corresponding monocyclic pyrrolyl



Scheme 1

[+] Respectfully and with best regards dedicated to Prof. Dr. *W. Wiegrebe*, Regensburg, on the occasion of his 60th birthday.

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Table 1. Inhibition of bovine cyclooxygenase and 5-lipoxygenase by pyrrole derivatives **4** and **8**

	% inhibition, conc. 10 μM (3,3 μM)	
Compound	**CO**	**5-LO**
4a	16	0
4b	40	35
4c	*	48
4d	72	78
4e	84	94 (27)
4f	*	99 (56)
4g	84	99 (60)
4h	71	85
4i	69	97 (47)
Indomethacin	(100)	-
NDGA	-	(100)
8a	19	23
8b	*	28
8c	18	20
8d	*	31
8e	17	48
8f	*	72
8g	32	82
8h	0	48
8i	0	55
8j	48	90

* Superimposed peaks of 12-HHT and compound. The values represent the average of two determinations (range < 10%).

Table 2. *N*-Alkyl pyrroles **2** and **6**

Compound	yield (%)	mp (°C)	formula (m_r)
2b	53	92-93[7]	$C_{18}H_{17}N$ (247,3)
2c	61	oil	$C_{19}H_{19}N$ (261,4)
2d	65	80	$C_{20}H_{21}N$ (275,4)
2e	66	oil	$C_{21}H_{23}N$ (289,4)
2f	*	*	$C_{22}H_{25}N$ (303,4)
2g	*	*	$C_{23}H_{27}N$ (317,5)
2h	26	116	$C_{21}H_{23}N$ (289,4)
2i	51	132	$C_{22}H_{25}N$ (303,4)
6b	52	149	$C_{18}H_{17}N$ (247,3)
6c	71	68-69	$C_{19}H_{19}N$ (261,4)
6d	57	oil	$C_{20}H_{21}N$ (275,4)
6e	55	oil	$C_{21}H_{23}N$ (289,4)
6f	59	oil	$C_{22}H_{25}N$ (303,4)
6g	40	oil	$C_{23}H_{27}N$ (317,5)
6h	*	*	$C_{21}H_{23}N$ (289,4)
6i	16	107	$C_{22}H_{25}N$ (303,4)
6j	34	99	$C_{23}H_{27}N$ (317,5)

* These compounds were used without further purification

systems part of our interest. In the present study the *in vitro* cyclooxygenase and 5-lipoxygenase inhibitory potencies of various *N*-alkyl diphenyl pyrrolyl acetic and propionic acids were investigated.

Chemistry

The pyrrolyl propionic and acetic acid derivatives **4** and **8**, resp., were prepared as outlined in Scheme 1, by phase transfer (PTC) catalyzed *N*-alkylation[4], reaction with methyl acrylate or ethyl diazoacetate, followed by alkaline hydrolysis of the esters **3** and **7**. 2-Methyl-3,4-diphenylpyrrole (**1**) and 5-methyl-2,3-diphenylpyrrole (**5**) were synthesized according to[5]. Instead of a *p*-toluene sulfonic ester or an alkyl bromide and PTC neopentyl bromide in DMSO at 130-140°C was used to obtain **2i** and **6i**, respectively. The structures of all compounds **2-8** were verified by their analytical and spectral data. The substitution at the N-atom and

Table 3. Methyl pyrrolyl propionate derivatives **3** and ethyl pyrrolyl acetate derivatives **7**

Compound	yield (%)	formula (m_r)
3a	44	$C_{21}H_{21}NO_2$ (319,4)
3b	60	$C_{22}H_{23}NO_2$ (333,4)
3c	49	$C_{23}H_{25}NO_2$ (347,5)
3d	64	$C_{24}H_{27}NO_2$ (361,5)
3e	56	$C_{25}H_{29}NO_2$ (375,5)
3f	67	$C_{26}H_{31}NO_2$ (389,5)
3g	43	$C_{27}H_{33}NO_2$ (403,6)
3h	53	$C_{25}H_{29}NO_2$ (375,5)
3i	46	$C_{26}H_{31}NO_2$ (389,5)
7a	33	$C_{21}H_{21}NO_2$ (319,4)
7b	42	$C_{22}H_{23}NO_2$ (333,4)
7c	51	$C_{23}H_{25}NO_2$ (347,5)
7d	33	$C_{24}H_{27}NO_2$ (361,5)
7e	38	$C_{25}H_{29}NO_2$ (375,5)
7f	27	$C_{26}H_{31}NO_2$ (389,5)
7g	45	$C_{27}H_{33}NO_2$ (403,6)
7h	32	$C_{25}H_{29}NO_2$ (375,5)
7i	43	$C_{26}H_{31}NO_2$ (389,5)
7j	30	$C_{29}H_{37}NO_2$ (431,6)

* Except **3i**, mp. 104°C, all the products are colorless oils purified by column chromatography (see Exp. Part).

Table 4. Pyrrolyl propionic acid derivatives **4** and pyrrolyl acetic acid derivatives **8**

Compound	yield (%)	mp (°C)	formula (m_r)
4a	40	60	$C_{20}H_{19}NO_2$(305,4)
4b	59	108	$C_{21}H_{21}NO_2$(319,4)
4c	56	157	$C_{22}H_{23}NO_2$(333,4)
4d	68	128	$C_{23}H_{25}NO_2$(347,5)
4e	49	49-51	$C_{24}H_{27}NO_2$(361,5)
4f	56	oil	$C_{25}H_{29}NO_2$(375,5)
4g	77	oil	$C_{29}H_{31}NO_2$(389,5)
4h	79	50-51	$C_{24}H_{27}NO_2$(361,5)
4i	62	48-49	$C_{25}H_{29}NO_2$(375,5)
8a	26	110	$C_{19}H_{17}NO_2$(291,3)
8b	60	161	$C_{20}H_{19}NO_2$(305,4)
8c	63	177	$C_{21}H_{21}NO_2$(319,4)
8d	65	172	$C_{22}H_{23}NO_2$(333,4)
8e	58	129	$C_{23}H_{25}NO_2$(347,5)
8f	69	121	$C_{24}H_{27}NO_2$(361,5)
8g	71	127	$C_{25}H_{29}NO_2$(375,5)
8h	72	168	$C_{23}H_{25}NO_2$(347,5)
8i	74	163	$C_{24}H_{27}NO_2$(361,5)
8j	58	45	$C_{27}H_{33}NO_2$(403,6)

the pyrrole nucleus, resp., was proved on the basis of the NMR-spectra of **1** and **5**.

Results and discussion

Inhibition of 5-lipoxygenase was determined by monitoring the formation of leucotriene B4 (LTB4) in bovine polymorphonuclear leucocytes (PMNLs), 12-hydroxyheptadecatrienoic acid (12-HHT) formed in bovine platelets was used as an indicator of the cyclooxygenase activity. For details concerning the testsystem, *e.g.* cell preparations, incubation procedures, sample preparations, reverse-phase HPLC on analysis statistics and validity of results, see Experimental protocols and reference[6]. Indomethacin and

nordihydroguaiaretic acid (NDGA) were applied as reference drugs.

All the pyrrolyl propionic and acetic acid derivatives **4** and **8**, respectively, were tested for their ability to inhibit bovine cyclooxygenase and 5-lipoxygenase. As shown in Tab. 1 some of the compounds tested revealed significant inhibitory activity at the given doses. In particular, the highest inhibition was exhibited by the propionic acid derivative **4g** which prevented the formation of LTB4 by 99% and of 12-HHT by 84% at 10 μM, the approximate IC 50 (5-LO) was 2.5 μM. In the same experimental conditions the reference NDGA (3.3 μM) inhibited LTB4 production by 100%, indomethacin (3.3 μM) showed an inhibition of the cyclooxygenase by 100%, too. A C_4-C_6-side chain (**4e-4g**) at the N-atom seems to be favorable for optimal activity whereas branched alkyl substituents decreased the potency as shown by comparison of **4e/4h** and **4f/4i**. On account of higher lipophilicity the inhibition of the 5-lipoxygenase increased linearly from **4b** to **4g**. Concerning both enzymes compounds with a NH function (**4a**, **8a**) have low inhibitory potencies or were completely inactive.

In general, pyrrolyl acetic acid derivatives **8** are less potent inhibitors of the bovine cyclooxygenase and lipoxygenase, respectively. For a better understanding of structure activity phenomena the regioisomeric 2-(2-methyl-3,4-diphenyl-pyrrol-5-yl) acetic acids would be optimal but due to rapid decarboxylation these experiments were not performed. **8j** with R = C_8H_{17} is the most active compound in this series. All derivatives are better 5-lipoxygenase than cyclooxygenase inhibitors.

Summarizing, most of the tested compounds showed significant inhibitory activity, except those with a NH-function or R = CH_3, C_2H_5, and a selectivity for inhibiting the 5-lipoxygenase of bovine PMNLs. The highest degree of

Table 5. IR and ^{1}H-NMR data of pyrrolyl propionic acid derivatives **4** and pyrrolyl acetic acid derivatives **8**

Compound	IR (cm^{-1};KBr)	^{1}H-NMR (90 MHz, $CDCl_3$/TMS, δ (ppm))
4a	3650-2300 (OH) 3400 (NH), 1700 (C=O)	2.29 (s, 3H, Pyr-CH_3), 2.54-2.81 (m, 2H, -CH_2CO-), 2.81-3.07 (m, 2H, Pyr-CH_2-), 6.88-7.36 (m, 10H, Arom.), 8.32 (s, 1H, >NH)
4b	3400-2400 (OH), 1700 (C=O)	2.27 (s, 3H, Pyr-CH_3), 2.40-2.67 (m, 2H, -CH_2CO), 2.90-3.13 (m, 2H, Pyr-CH_2-), 3.56 (s, 3H, >N-CH_3), 6.93-7.37 (m, 10H, Arom.)
4c	3300-2400 (OH), 1715 (C=O)	1.38 (t, 3H, J=7 Hz, -CH_3), 2.29 (s, 3H, Pyr-CH_3), 2.37-2.63 (m, 2H, -CH_2CO-), 2.89-3.14 (m, 2H, Pyr-CH_2-), 3.80-4.14 (q, 2H, J=7 Hz, >N-CH_2-), 6.91-7.32 (m, 10H, Arom.)
4d	3300-2400 (OH), 1705 (C=O)	1.02 (t, 3H, J=7 Hz, -CH_3), 1.53-1.96 (m, 2H, -CH_2) 2.27 (s, 3H, Pyr-CH_3), 2.34-2.62 (m, 2H, -CH_2CO-), 2.86-3.15 (m, 2H, Pyr-CH_2-), 3.66-3.98 (m, 2H, >N-CH_2-), 6.97-7.34 (m, 10H, Arom.)
4e	3400-2400 (OH), 1715 (C=O)	1.00 (t, 3H, J=7 Hz, -CH_3), 1.18-1.88 (m, 4H, -CH_2) 2.27 (s, 3H, Pyr-CH_3), 2.36-2.63 (m, 2H, -CH_2CO-), 2.85-3.14 (m, 2H, Pyr-CH_2-), 3.70-4.00 (m, 2H, >N-CH_2-), 6.90-7.33 (m, 10H, Arom.)
4f	3400-2400 (OH), 1715 (C=O) (film)	0.80-1.00 (m, 3H, -CH_3), 1.22-1.58 (m, 4H, -CH_2) 1.58-1.97 (m, 2H, >N-CH_2-), 2.27 (s, 3H, Pyr-CH_3), 2.37-2.67 (m, 2H, -CH_2CO-), 2.86-3.17 (m, 2H, Pyr-CH_2-), 3.72-4.03 (m, 2H, >N-CH_2-), 6.93-7.37 (m, 10H, Arom.)
4g	3600-2300 (OH), 1715 (C=O) (film)	0.78-1.10 (m, 3H, -CH_3), 1.17-1.55 (m, 6H, -CH_2) 1.56-2.00 (m, 2H, >N-CH_2-CH_2-), 2.26 (s, 3H, Pyr-CH_3), 2.35-2.68 (m, 2H, -CH_2CO-), 2.80-3.16 (m, 2H, Pyr-CH_2), 3.70-4.00 (m, 2H, >N-CH_2-), 6.93-7.32 (m, 10H, Arom.)
4h	3200-2400 (OH), 1715 (C=O)	0.98 (d, 6H, J=7 Hz, -CH_3), 1.83-2.23 (m, 1H, -CH<), 2.25 (s, 3H, Pyr-CH_3), 2.30-2.58 (m, 2H, -CH_2CO-), 2.84-3.15 (m, 2H, Pyr-CH_2), 3.68 (d, 2H, J=7 Hz, >N-CH_2-), 6.93-7.37 (m, 10H, Arom.)
4i	3400-2400 (OH), 1710 (C=O)	1.04 (s, 9H, -CH_3), 2.26 (s, 3H, Pyr-CH_3), 2.21-2.49 (m, 2H, -CH_2CO-), 2.93-3.20 (m, 2H, Pyr-CH_2-), 3.76 (s, 2H, >N-CH_2-), 6.92-7.33 (m, 10H, Arom.)
8a	3600-2400 (OH) 3400 (NH), 1710 (C=O)	2.30 (s, 3H, Pyr-CH_3), 3.39 (s, 2H, -CH_2CO-), 7.00-7.37 (m, 10H, Arom.), 8.01 (s, 1H, <NH)
8b	3300-2400 (OH) 1705 (C=O)	2.30 (s, 3H, Pyr-CH_3), 3.44 (s, 3H, >N-CH_3), 3.49 (s, 2H, -CH_2CO-), 6.93-7.38 (m, 10H, Arom.)

Table 5 cont.

8c	3300-2400 (OH) 1705 (C=O)	1.20 (t, 3H, J=7 Hz, $-CH_3$), 2.30 (s, 3H, Pyr-CH_3), 3.49 (s, 2H, $-CH_2CO-$), 3.83 (q, 2H, J=7 Hz, >N-CH_2-), 6.96-7.34 (m, 10H, Arom.)
8d	3240-2300 (OH) 1700 (C=O)	0.76 (t, 3H, J=7 Hz, $-CH_3$), 1.35-1.85 (m, 2H, >N-CH_2-CH_2-), 2.30 (s, 3H, Pyr-CH_3), 3.49 (s, 2H, $-CH_2CO-$), 3.61-3.89 (m, 2H, >N-CH_2-), 6.92-7.33 (m, 10H, Arom.)
8e	3240-2300 (OH) 1705 (C=O)	0.65 -0.93 (m, 3H, $-CH_3$), 0.93-1.35 (m, 2H, $-CH_2$-), 1.35-1.78 (m, 2H, >N-CH_2-CH_2-), 2.30 (s, 3H, Pyr-CH_3), 3.50 (s, 2H, $-CH_2CO-$), 3.63-3.93 (m, 2H, >N-CH_2-), 6.94-7.34 (m, 10H, Arom.)
8f	3240-2300 (OH) 1710 (C=O)	0.67 -0.97 (m, 3H, $-CH_3$), 0.97-1.35 (m, 4H, $-CH_2$-), 1.35-1.83 (m, 2H, >N-CH_2-CH_2-), 2.30 (s, 3H, Pyr-CH_3), 3.49 (s, 2H, $-CH_2CO-$), 3.61-3.91 (m, 2H, >N-CH_2-), 6.96-7.36 (m, 10H, Arom.)
8g	3240-2300 (OH) 1710 (C=O)	0.71 -0.99 (m, 3H, $-CH_3$), 0.99-1.40 (m, 6H, $-CH_2$-), 1.40-1.81 (m, 2H, >N-CH_2-CH_2-), 2.30 (s, 3H, Pyr-CH_3), 3.50 (s, 2H, $-CH_2CO-$), 3.62-3.91 (m, 2H; >N-CH_2-), 6.98-7.36 (m, 10H, Arom.)
8h	3260-2300 (OH) 1710 (C=O)	0.63 (d, 6H, J=7 Hz, $-CH_3$), 1.47-1.89 (m, 1H, -CH<), 2.29 (s, 3H, Pyr-CH_3), 3.50 (s, 2H, $-CH_2CO-$), 3.71 (d, 2H, J=8 Hz, >N-CH_2-), 6.98-7.33 (m, 10H, Arom.)
8i	3300-2300 (OH) 1710 (C=O)	0.64 (s, 9H, $-CH_3$), 2.30 (s, 3H, Pyr-CH_3), 3.47 (s, 2H, $-CH_2CO-$), 3.90 (m, 2H, >N-CH_2-), 6.93-7.31 (m, 10H, Arom.)
8j	3300-2400 (OH) 1715 (C=O)	0.74 -1.00 (m, 3H, $-CH_3$), 1.00-1.36 (m, 10H, $-CH_2$-), 1.36-1.79 (m, 2H, >N-CH_2-CH_2-), 2.30 (s, 3H, Pyr-CH_3), 3.50 (s, 2H, $-CH_2CO-$), 3.62-3.95 (m, 2H, >N-CH_2-), 6.99-7.35 (m, 10H, Arom.)

activity was found in acid **4g**, however, it was less potent than comparable dihydropyrrolizinyl derivatives[3] and the reference compounds NDGA and indomethacin, respectively. Further investigations to optimize the inhibitory profile are under work.

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Experimental Part

Chemistry

Melting points: Büchi 510 apparatus, uncorrected.- IR spectra: Beckman Acculab III, KBr disks (unless otherwise stated).- ^{1}H-NMR spectra: Varian EM 390 (90 MHz) instrument in $CDCl_3$ (unless otherwise stated) using TMS as the internal standard.- Analyses indicated by the elemental symbols were within ± 0.4% of the theoretical values.- All reagents used were of analytical grade and obtained as follows: salts for buffer solutions, solvents: Merck, Darmstadt (Germany); calcium ionophore A 23187, NDGA, indomethacin: Sigma, München (Germany). HPLC reference substances LTB_4, 12-HHT, HPLC internal standards PGB_2 and 15-keto-PGE_2: Paesel, Frankfurt/M. (Germany). Bovine blood was obtained from the local slaughterhouse.

The pyrroles **1** and **5** were synthesized according to[5].

General procedure for the synthesis of **2** *and* **6**

To a solution of 2 mmol pyrrole **1** and **5**, resp., 2.2 mmol methyl *p*-toluenesulfonate (**2b**, **6b**) or the corresponding alkyl bromide (except **2i**, **6i**) and 1 mmol of tetrabutylammonium bromide in 10 ml ether, 5 ml of 50% aqueous NaOH were added. The mixture was refluxed and stirred for 8 h. After addition of 100 ml water the solution was extracted twice with ether. The combined ether layers were washed with 1% H_3PO_4 and water. The solvent was removed and the crude products purified by column chromatography on Al_2O_3 neutral, activity grade II, with n-hexane/ether 9/1 (v/v). Reaction of the appropiate pyrrole with 3 mmol neopentyl bromide and 2.6 mmol Na-t-butoxide in 6 ml absol. DMSO at 130-140°C for 45 min led to **2i** and **6i**, resp. (Table 2).

General procedure for the synthesis of methyl 3-(5-methyl-3,4-diphenylpyrrol-2-yl)propionates **3**

A mixture of 1 mmol **2a-i**, 1.5 mmol methyl acrylate and 0.06 ml BF_3/ether complex in 4 ml absol. CH_2Cl_2 was stirred for 1 h at room temp. The reaction was quenched with 50 ml H_2O and the solution extracted twice with ether. The products obtained from the org. layer were purified by column chromatography on neutral Al_2O_3, activity grade II with n-hexane/ether 1/4 (v/v) (Table 3).

General procedure for the synthesis of ethyl (2-methyl-4,5-diphenylpyrrol-3-yl)acetates **7**

At 110°C to a solution of 2.5 mmol **6a-j** in 2.5 mmol absol. toluene 3.75 mmol ethyl diazoacetate in 2 ml absol. toluene were added together with small portions of copper powder. After 1 h copper was filtered off and the solvent was evaporated. The residue was purified by column chromatography on SiO_2 with n-hexane/ether 1/1 (v/v) (Table 3).

General procedure for the preparation of the acids **4** *and* **8**

Each of the esters (0.4 mmol) **3** and **7**, resp., in 3 ml EtOH and 2 ml of 10% aqueous KOH were refluxed for 1 h. After cooling the solution was

poured into 100 ml 5% aqueous NaCl and extracted twice with 50 ml Et_2O. The aqueous layer was acidified with diluted H_3PO_4 and extracted with ether. From the combined ether layers the products precipitated after addition of n-hexane or were purified by column chromatography on SiO_2 with n-hexane/ether 1/1 (v/v) (tables 4 and 5).

Enzyme assays

Cell preparations and apparatus see[6]

5-LO assay: A sample of dimethyl sulphoxide (DMSO) solution of drug, 2.5 µl, and 0.8 ml of the bovine PMNL suspension were preincubated in ground glass borosilicate tubes for 10 min at 37°C, and after 5 min, 0.2 ml $CaCl_2$-solution (10 mM in 0.8% w/v saline) was added. The cells were stimulated by adding 2.5 µl of a DMSO solution of calcium ionophore A 23187 (4.2 mg/ml = final concentration of 20 µM) and the incubation was continued for 5 min at 37°C. Arachidonic acid (AA) metabolism was terminated by the addition of 1 ml of a mixture of acetonitrile and methanol (1:1 v/v) containing 0.2 µg PGB_2 as internal standard and 6 µg NDGA as oxygen scavenger. The glass tubes were cooled for 20 min in an ice bath and then centrifuged at 4000 g for 15 min at 0°C. The supernatants were stored at -20°C.

Cyclooxygenase assay was performed using 12-HHT as indicator of enzyme activity. The test was carried out under the conditions described for the 5-LO assay (see above) except for the application of 0.8 ml bovine platelet suspension instead of PMNL suspension and the use of 3-(2,2-dimethyl-5,6-diphenyl-2,3-dihydro-1*H*-pyrrolizin-7-yl)-butyric acid[1] (0.47 µg per ml acetonitrile/methanol solution) as internal standard instead of PGB_2 and incubation for 1 min instead of 5 min after calcium ionophore A 23187 stimulation. Using exogenous AA, 50 µl of a sodium arachidonate solution (0.14 mg/ml phosphate buffered saline = final concentration 20 µM) was added instead of calcium ionophore A 23187 solution.

Sample preparation

5-LO assay: The supernatants were diluted with 10 ml of water and the AA metabolites isolated with octadecyl reverse-phase extraction columns following the procedure of *Verhagen*[8]. The eluant (3 ml) was diluted with 3 ml of water and then subjected to HPLC.

CO assay using 12-HHT as indicator of enzyme activity: Sample preparation was carried out as described for the 5-LO assay.

Reverse-phase HPLC analysis: 2 ml samples were injected on a Nucleosil 7 C_{18} column (500 mg, 6 ml, Baker, Groß Gerau, Germany). After each run the column was washed twice with 2 ml of methanol. The AA metabolites were quantified by their peak area ratio. The absolute amount of the enzyme products was calculated using the molar absorption coefficients. For further details see [6].

References

1 Part XVI: G. Dannhardt, M. Lehr, *Chemiker-Ztg.* **1990**, *114*, 244-246.

2 T.Y. Shen, *Drugs Exptl. Clin. Res.* **1977**, 2, 1-8.
H. Fenner, *Dtsch. Apoth. Ztg.* **1984**, *124*, 1257-1264.

3 G. Dannhardt, M. Lehr, *Arch. Pharm. (Weinheim)* **1988**, *321*, 545-549.
G. Dannhardt, M. Lehr, *Arch. Pharm. (Weinheim)* **1988**, *321*, 159-162 and unpublished results.

4 N.C. Wang, K.E. Teo, H.J. Anderson, *Can. J. Chem.* **1977**, *55*, 4112-4116.

5 R.W. Guy, R.A. Jones, *Aust. J. Chem.* **1966**, *19*, 1871-1885.

6 G. Dannhardt, M. Lehr, *J. Pharm. Pharmacol.* **1992**, *44*, 419–424.

7 I. Adachi, K. Harada, H. Kano, *Tetrahedron Lett.* **1969**, *55*, 4875-4878.

8 J. Verhagen, G.A. Wassink, G.M. Kijne, R.J. Victor, P.L.B. Bruynzeel, *J. Chromatogr.*, **1986**, *378*, 208-214.

[Ph30]