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Original article

Cyclooxygenase-1/2 (COX-1/COX-2) and 5-lipoxygenase (5-LOX) inhibitors of the 6,7-diaryl-2,3-1*H*-dihydropyrrolizine type

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Abstract

A series of 6,7-diaryl-2,3-1*H*-dihydropyrrolizines was prepared as COX-1/COX-2 and 5-LOX inhibitors. The inhibition of COX-1 was evaluated using intact bovine platelets as the enzyme source, whereas LPS-stimulated human monocytes served as the enzyme source for inducible COX-2. The determination of arachidonic metabolites was performed by HPLC for COX-1 and RIA for COX-2. The balance between COX-1/COX-2 and 5-LOX inhibition can be shifted by modifying the substitution pattern of the phenyl moiety at the 6- and 7-position of the pyrrolizine nucleus. Structure–activity relationships are discussed.

Keywords: Non-steroidal anti-inflammatory drugs; Diarylpyrrolizines; COX-1, COX-2 and 5-LOX inhibition; Structure–activity relationship

1. Introduction

NSAIDs are widely used for the treatment of pain, inflammation and fever. The main mechanism of action of these drugs is believed to be the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) [1] and consecutively the conversion of arachidonic acid to prostaglandins.

The major side effects associated with all the currently available NSAIDs are gastrointestinal (GI) hemorrhagia and ulceration [2]. These side effects during anti-inflammatory therapy are caused by interference with the physiological properties of prostaglandins. Recently several approaches have been proposed to reduce the unwanted side effects of NSAIDs. Among these, one involves the selective inhibition of COX isoenzymes; COX-1 is constitutively expressed and probably plays a role as a 'housekeeping enzyme', for example in maintaining the lining of the stomach and in endothelial cells contributing to the normal function of the cardiovascular system via the release of prostacyclin (PGI_2). COX-2, on the other hand, is induced by many kinds of inflammatory mediators and plays an important role in the prostaglandin biosynthesis associated with inflammatory responses. Therefore, NSAIDs with selective COX-2 inhibitory activity should be more useful for the treatment of inflammatory diseases. However, the simple concept of COX-2 being an exclusively pro-inflammatory inducible enzyme can no longer be upheld. Recently, it was found that COX-2 is expressed constitutively in the brain, spinal cord and kidney, as well as in numerous other organs [3]. It was hypothesised that selective inhibition of COX-2, may—through inhibition of endothelial prostacyclin synthesis—have severe side effects in the cardiovascular system and promote thrombogenesis [4,5]. Selective COX-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adherence to the endothelium [6].

After the introduction of the highly selective COX-2 inhibitors celecoxib (Celebrex®) and rofecoxib (Vioxx®; Fig. 1) onto the market, the VIOXX gastrointestinal Outcomes Research (VIGOR) and the Celecoxib long-term Arthritis Safety Study (CLASS) studies suggest that these agents causing also GI injury like conventional NSAIDs and increased systemic blood pressure [7]. A more recent strategy for devising a gastric-sparing

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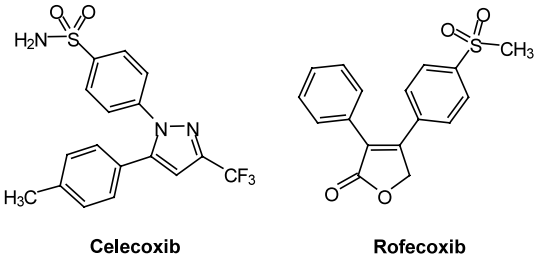


Fig. 1. Structures of selective COX-2 inhibitors.

NSAID involves chemically coupling a nitric oxide (NO)-releasing moiety to the parent NSAID [8]. Along with prostaglandins NO appears to play an important cytoprotective role in GI homeostasis [9].

Another interesting class of compounds to minimise the toxicity of NSAIDs are dual COX/LOX-inhibitors.

Besides interfering with the production of prostaglandins these substances inhibit the biosynthesis of chemotactic leukotrienes, which are another important mediator in inflammatory processes, i.e. they induce the invasion of neutrophils into the inflamed area as a prerequisite for the formation of gastric ulcers [10].

Diarylpyrrolizines have been widely investigated inhibitors of COX-1, COX-2 and 5-LOX pathways. A compound of these series Licofelone (ML-3000; Fig. 2) showed anti-inflammatory and analgesic activity in osteoarthritis in clinical trials comparable to conventional NSAIDs and selective COX-2 inhibitors with a safer GI profile [11–15]. COX-1/COX-2 inhibitors with balanced inhibiting capacities (Gkz) on both enzymes could also improve the therapeutic benefit in the treatment of inflammatory diseases [16]. 7-*tert*-Butyl-2,3-dihydro-3,3-Dimethyl derivatives and 1,2-isothiazolidine-1,1-dioxide (γ-sultam) derivatives (S-2474) (Fig. 2) were recently reported as COX-2/5-LOX-inhibitors. One of these substances was found to be the active

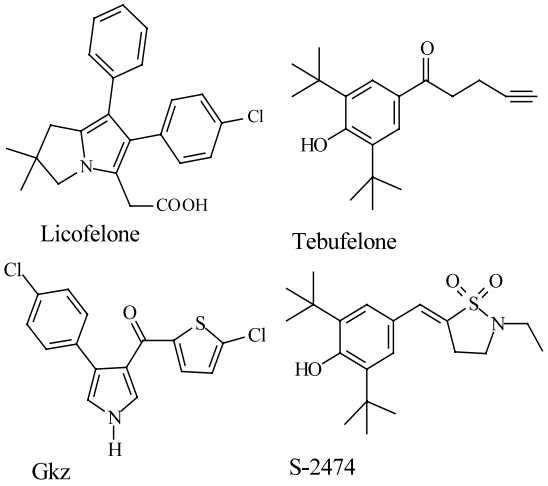


Fig. 2. Structures of a well balanced COX-1/2 and dual inhibitors of COX-2/5-LOX (for explanations see text).

metabolite of tebufelone, which acts as an antioxidant [17,18].

This paper deals with diarylpyrrolizines as a class of compounds with multiple inhibitory effects on both COX enzymes, especially COX-2, and 5-LOX, which may display therapeutic advantages over NSAIDs in treating anti-inflammatory diseases as discussed above.

2. Chemistry

The synthetic route is analogous to that of Dannhardt [19] and is outlined in Fig. 3. 4-Chloro-butyronitril was condensed with a commercially available benzyl-Grignard and ring closure to 2-benzyl-Δ^1-pyrroline was accomplished in situ by heating. The Δ^1-pyrrolines were cyclised with the corresponding bromoacetophenones (hetero-aromatic 1-bromo-1-ethanones) in abs. CH_2Cl_2 by stirring for 24 h at room temperature. Pyrrolinium intermediates were not isolated. Then a solution (5%) of $NaHCO_3$ was added and stirring continued for a further 24 h. After addition of water, the organic layer was separated and dried (Na_2SO_4 sicc.) and the solvent evaporated. Variation at the substitution pattern was accomplished by using different kinds of 2-benzyl-Δ^1-pyrrolines and bromoacetophenones (hetero-aromatic 1-bromo-1-ethanones). The data of the compounds are summarised in Tables 3 and 4.

3. Biological assays

All the compounds were tested in intact cell assays described earlier [19–22].

4. Results and discussion

Fig. 3 shows the synthetic pathway of the general procedure.

First all the compounds were tested for their ability to inhibit COX-1 and 5-LOX. The most interesting compounds underwent further investigation to ascertain their COX-2 inhibiting activity. Table 1 summarises the results of the compounds tested.

4.1. 6,7-Diaryl-2,3-dihydropyrrolizines

The derivatives **1**–**9** are different in R^1 and R^2. Table 1 summarises the impact of these substituents. All the compounds except **7** inhibit the COX-1 pathway. The potent COX-1 inhibiting substances **1** and **8** bear a methyl sulphide group at the C-6 phenyl moiety with an approximate IC_{50} of 1.1 versus 0.03 μM. The most potent COX-1 inhibitor **8** bears an additional lipophilic substituent R^2. The thioether compounds may act as

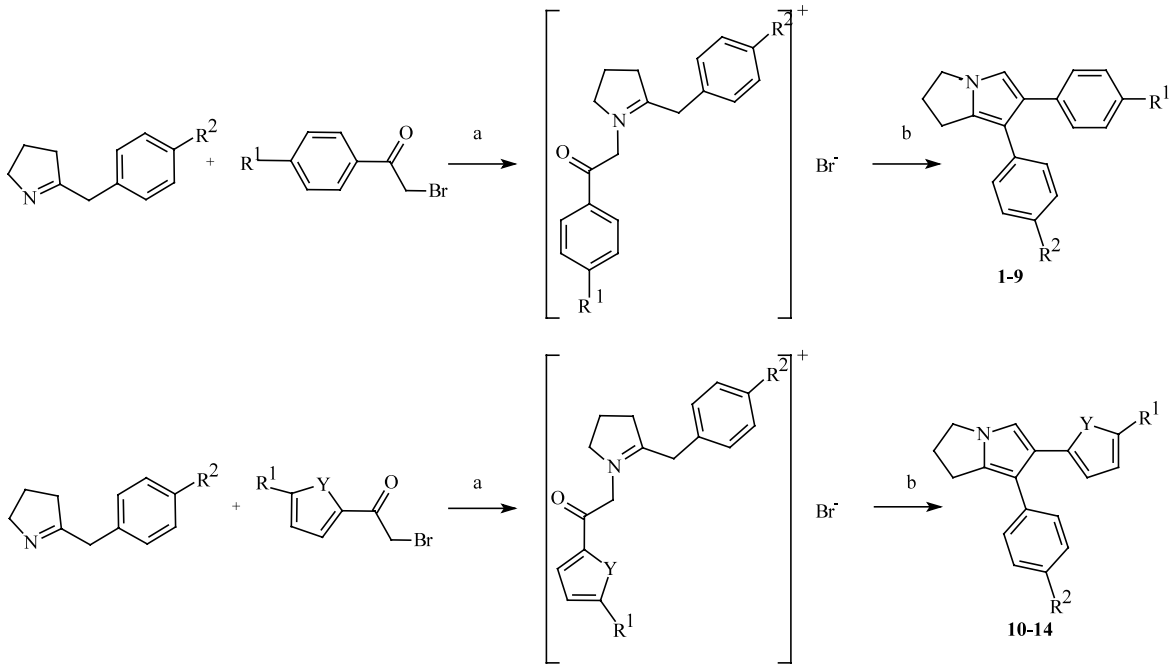
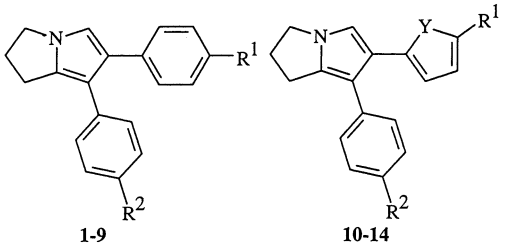


Fig. 3. Synthetic pathway. (a) $CH_2Cl_{2(abs.)}$ room temperature 24 h. (b) $NaHCO_3$ (5%), 24 h.; for R^1, R^2 and Y see Table 1.

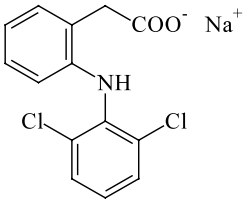
Table 1
In vitro inhibitory potencies of the 6,7-diaryl-2,3-dihydropyrrolizines and the 6-heteroaryl-7-aryl-2,3-dihydropyrrolizines



Compound	R^1	R^2	Y	IC_{50} (μM) COX-1 [a]	IC_{50} (μM) 5-LOX [a]	IC_{50} (μM) COX-2 [a]	IC_{50} COX-1/IC_{50} COX-2 [a]
1	SCH_3	H	–	1.1	0%	30% (1 μM)	n.d.
2	SO_2CH_3	H	–	25%	10	0.03	n.d.
3	$NHSO_2CH_3$	H	–	10%	3.4	3.6	n.d.
4	SCH_3	F	–	28%	0%	26% (0.1 μM)	n.d.
5	SO_2CH_3	F	–	4.3	0%	0.16	27
6	SO_2CH_3	Cl	–	8.2	0%	0.008	1025
7	$NHSO_2CH_3$	Cl	–	0%	0%	n.t.	n.d.
8	SCH_3	CH_3	–	0.03	0%	0.9	0.03
9	SO_2CH_3	CH_3	–	0.7	10	0.005	140
10	H	H	S	33%	10	20%	n.d.
11	Cl	H	S	3.9	0%	n.t.	n.d.
12	Br	H	S	25%	8.4	n.t.	n.d.
13	CH_3	H	S	0%	0%	1.8	n.d.
14	H	H	O	20%	10	n.t.	n.d.
Licofelone				0.16	0.21	0.37	0.43
Diclofenac				0.0028	n.t.	0.0004	7
Indomethacin				0.004	n.t.	0.0005	8
4″-Cl-SC 57666				> 10	n.t.	0.016	> 625

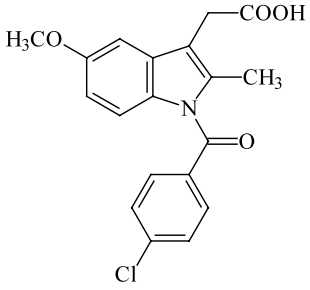
[a] IC_{50} values of compounds tested represent the mean value of four determinations (μM or percentage inhibition at a concentration of 10 μM), n.t., not tested; n.d., not determined.

Table 2
Chemical structures of diclofenac, indomethacin and cyclopentene derivative 4″-Cl-SC-57666, IC_{50} values, COX-1/COX-2 ratios



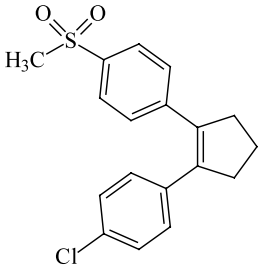
Diclofenac

IC_{50} values:
0.0028 μM (COX-1)
0.0004 μM (COX-2)
COX-1/COX-2 ratio = 7



Indomethacin

IC_{50} values:
0.004 μM (COX-1)
0.0005 μM (COX-2)
COX-1/COX-2 ratio = 8



4''-Cl-SC 57666

IC_{50} values:
> 10 μM (COX-1)
0.016 μM (COX-2)
COX-1/COX-2 ratio = > 625

peroxidase-reducing agents by consuming electrons and oxygen, hence destroying the peroxidase-derived oxidant, which is thought to oxidise the putative tyrosine residue to a tyrosyl radical, and thereby initiating the cyclooxygenase pathway [23]. Sulindac, an anti-inflammatory drug with a sulphoxide moiety has to be metabolised to the corresponding thioether in order to become a potent inhibitor of cyclooxygenase. It was shown that the lipophilicity of the thioethers is 1000-fold higher, thus demonstrating the requirements for lipophilic interactions of drug and enzyme.

Compound **1** ($R^2 = H$) and **8** ($R^2 = CH_3$) inhibit COX-2 almost equipotently (IC_{50}: ≈ 1 μM). A significant increase of COX-2 inhibition is observed for **4** ($R^2 = F$) (26% inhibition at 0.1 μM). Whereas all the thioethers (**1**, **4**, and **8**) show no inhibiting potency against 5-LOX, by oxidation of the sulphur atom to a sulphone it is possible to shift the specificity for 5-LOX as well as for COX-2. The methanesulphonamide **3** shows moderate dual inhibition of COX-2 and 5-LOX (IC_{50}: 3.6 vs. 3.4 μM). The sulphone **2** inhibits COX-2 potently (IC_{50}: 0.03 μM) and shows significant inhibition of 5-LOX (IC_{50}: 10 μM). Compound **5** possesses a COX-1/COX-2 ratio of 27. The selective COX-2 inhibitors **6** and **9** in this range are sulphones, which bear a lipophilic chloro- or methyl-moiety R^2 documenting a COX-1/COX-2 ratio of 1025–140.

Compound **9** combines the structural requirements for COX-1/2 and 5-LOX inhibition (IC_{50}: 0.7, 0.005 and 10 μM). Summarising these data, it can be stated that the combination of a methyl sulphonyl group or methanesulphonamide residue R^1 with a lipophilic moiety R^2 produces triple inhibitors of COX-1/2 and 5-LOX (**2**, **3** and **9**).

Table 3
Melting points, yields and analytical data of new compounds

Compound	Formula (all compound were analysed for C, H, N) [a]	Yield (%)	Melting point (°C)	R^1	R^2
1	$C_{20}H_{19}NS$	60	99	SCH_3	H
2	$C_{20}H_{19}NO_2S$	55	170	SO_2CH_3	H
3	$C_{20}H_{20}N_2O_2S$	47	168	$NHSO_2CH_3$	H
4	$C_{20}H_{18}FNS$	65	120	SCH_3	F
5	$C_{20}H_{18}FNO_2$	58	128	SO_2CH_3	F
6	$C_{20}H_{18}ClNO_2S$	60	146	SO_2CH_3	Cl
7	$C_{20}H_{19}ClN_2O_2S$	55	190	$NHSO_2CH_3$	Cl
8	$C_{21}H_{19}NS$	58	190	SCH_3	CH_3
9	$C_{21}H_{21}NO_2S$	61	155	SO_2CH_3	CH_3
10	$C_{17}H_{15}NS$	65	115	H	H
11	$C_{17}H_{14}ClNS$	59	87	Cl	H
12	$C_{17}H_{14}BrNS$	58	105	Br	H
13	$C_{18}H_{15}NS$	61	94	CH_3	H
14	$C_{17}H_{15}NO$	55	95	H	H

[a] Elemental analysis is within ±0.4% for elements indicated unless otherwise.

Table 4
Physical data of compounds synthesised

Compound	IR (KBr)	^{1}H-NMR (200 MHz), δ (ppm), *J* (Hz)/MS (EI, 70 eV): *m/z*
1	1600 (C=C), 1320, 1150 (SO_2–N)	2.53 (s, 3H, $-SCH_3$), 2.57–2.61 (m, 2H, H-2), 2.99 (t, 2H, H-1, *J* = 7.0), 4.03 (t, 2H, H-2, *J* = 7.0), 6.76 (s, 1H, vinyl-H), 7.12–7.27 (m, 9H, aromatic) 306/305 (32, 3%, $M^{+\bullet}$), 290 (11%, $C_{19}H_{16}NS$), 258 (2%, $C_{19}H_{16}NS$), 202.07 (2%, $C_{16}H_{10}$), 56 (12%, C_3H_6N)
2	1600 (C=C), 1320, 1160 (SO_2–N)	2.55–2.60 (m, 2H, H-2), 2.95 (t, 2H, H-1, *J* = 7.30), 3.07 (s, 3H, $-SO_2CH_3$), 4.03 (t, 2H, H-3, *J* = 7.30), 6.85 (s, 1H, vinyl.-H), 7.12–7.30 (m, 5H, aromatic), 7.45 (d, 2H, AA′BB′, *J* = 8.3 H-aromatic), 7.75 (d, 2H, AA′BB′, *J* = 8.3 H-aromatic) 339/338/337 (6, 22, 100%, $M^{+\bullet}$) 258 (11%, $C_{19}H_{16}N$), 181 (4%, $C_{13}H_{11}N$)
3	1600 (C=C), 1320, 1160 (SO_2–N)	2.49–2.60 (m, 2H, H-2), 2.92–2.99 (m, 2H, H-1), 3.06 (s, 3H, $-CH_3$), 4.04 (t, 2H, H-3, *J* = 7.0), 6.50 (s, 1H, –NH), 6.74 (s, 1H, vinyl-H), 7.04–7.24 (m, 9H, aromatic) 355/354/353 (2, 6, 24%, $M^{+\bullet}$), 272 (100%, $C_{19}H_{16}N_2$)
4	1640 (C=C)	2.51 (s, 3H, $-SCH_3$), 2.57–2.65 (m, 2H, H-2), 2.99 (t, 2H, H-1, *J* = 7.0), 4.05 (t, 2H, H-2, *J* = 7.0), 6.77 (s, 1H, vinyl-H), 6.99–7.24 (m, 8H, aromatic) 323 (100%, $M^{+\bullet}$), 304 (14%, $M^{+\bullet}-F$), 276 (1%, $C_{19}H_{15}NF$)
5	1600 (C=C), 1320, 1160 (SO_2–N)	2.45–2.60 (m, 2H, H-2), 2.95 (t, 2H, H-1, *J* = 7,3), 3.08 (s, 3H, SO_2CH_3, *J* = 7,3), 6.88 (s, 1H, vinyl-H), 6.90–7.20 (m, 4H, aromatic), 7.38 (d, 2H, AA′BB′, *J* = 8.3, H-aromatic), 7.78 (d, 2H, AA′BB′, *J* = 8.3, H-aromatic) 356/355/354 (49, 100, 20, $M^{+\bullet}$), 276 (11%, $C_{19}H_{15}NF$), 248 (17%), 220 (7%)
6	1600 (C=O), 1320, 1160 (SO_2–N)	2.45–2.65 (m, 2H, H-2), 2.98 (t, 2H, H-1, *J* = 7,3), 3.10 (s, 3H, SO_2CH_3), 4.13 (t, 2H, H-3, *J* = 7.3), 6.85 (s, 1H, vinyl-H), 7.10 (d, 2H, AA′BB′, *J* = 8.3), 7.26 (d, 2H, AA′BB′, *J* = 8.3), 7.38 (d, 2H, AA′BB′, *J* = 8.3), 7.78 (d, 2H, AA′BB′, *J* = 8.3) 373/372/371 (38, 25, 100%, $M^{+\bullet}$), 292 (9% $C_{19}H_{15}NCl$), 257 (7%, $C_{19}H_{15}N$), 215 (2%, $C_{16}H_9N$)
7	1590 (C=O), 1320, 1160 (SO_2–N)	2.49–2.65 (m, 2H, H-2), 2.92–3.01 (m, 2H, H-1), 3.09 (s, 3H, $-CH_3$), 4.03 (t, 2H, H-3), 6.38 (s, 1H, –NH), 6.74 (s, 1H, vinyl-H), 7.00–7.30 (m, 8H, aromatic)
8	1630 (C=C)	2.34 (s, 3H, $-CH_3$), 2.47 (s, 3H, $-SCH_3$), 2.58 (m, 2H, H-2), 3.01 (t, 2H, H-1, *J* = 7,3), 4.01 (t, 2H, H-3, *J* = 7,3), 6.75 (s,1H, vinyl-H), 7.08–7.24 (m, 8H, aromatic) 319/318 (100, 9%, $M^{+\bullet}$), 304 (23%, $C_{20}H_{18}NS$), 228 (2%, $C_{14}H_{14}NS$), 123 (2%, C_7H_7S), 105 (3%, C_7H_7N)
9	1590 (C=C), 1320, 1150 (SO_2–N)	2.36 (s, 3H, $-CH_3$), 2.50–2.65 (m, 2H, H-2), 3.00 (t, 2H, H-1, *J* = 7,3), 3.08 (s, 3H, $-SO_2CH_3$), 4.04 (t, 2H, H-3, *J* = 7.3), 6.85 (s, 1H, vinyl-H), 7.10 (s, 4H, H-aromatic), 7.40 (d, 2H, AA′BB′, *J* = 8.3), 7.75 (d, 2H, AA′BB′, *J* = 8.3). 352/351 (23%, 100% $M^{+\bullet}$), 272 (11%, $C_{20}H_{18}N$), 257 (4%, $C_{19}H_{15}N$), 215 (1%, $C_{16}H_9N$)
10	1600 (C=C)	2.44–2.60 (m, 2H, H-2), 2.98 (t, 2H, 4,03 (t, 2H, H-3, *J* = 8.3), 6.75 (d, 1H, H-3), 6.83 (s, 1H, vinyl-H), 6.95 (t, 1H, H-4), 7.10 (d, 1H, H-5), 7.18–7.45 (m, 5H, aromatic) 265/264/263 (100, 21, 5%, $M^{+\bullet}$), 239 (13%, $C_{15}H_{13}NS$), 188 (8%), 140 (3%, $C_{10}H_6N$)
11	1600 (C=C)	2.45–2.59 (m, 2H, H-2), 2.96 (t, 2H, H-1, *J* = 7.3), 4.01 (t, 2H, H-3, *J* = 7.3), 6.51 (d, 1H, H-3 thiophen), 6.72 (d, 1H, 4-H thiophen), 6,79 (s,1H, vinyl-H), 7.14–7.36 (m, 5H, aromatic) 299/298/297 (100, 12, 3%, $M^{+\bullet}$), 264 (3%, $C_{17}H_{14}NS$), 207 (3%, $C_{15}H_{13}N$), 165 (3%, $C_{12}H_7N$)
12	1600 (C=C)	2.43–2.60 (m, 2H, H-2), 2.97 (t, 2H, H-1, *J* = 7.3), 4.00 (t, 2H, H-3, *J* = 7.3), 6.51 (d, 1H, H-3 thiophen), 6.79 (s 1H, vinyl-H), 6.84 (d, 1H, H-4 thiophen), 7.14–7.36 (m, 5H, aromatic) 345/344/343 (11, 65, 20%, $M^{+\bullet}$), 265 (100%, $C_{17}H_{14}NS$), 236 (16%, $C_{15}H_{10}NS$), 188 (18%), 146 (16%), 90.9 (17%)
13	1595 (C=C)	2.44 (s, 3H, $-CH_3$), 2.50–2.65 (m, 2H, H-2), 3.00 (t, 2H, H-1, *J* = 7.3), 4.05 (t, 2H, H-3, *J* = 7.3), 6.48–6.70 (m, 2H, H-3, H-4, thiophen), 6.81 (s, 1H, vinyl-H), 7.14–7.5 (m, 5H, aromatic) 2.43–2.57 (m, 2H, H-2), 2.87–3.05 (t, 2H, H-1, *J* = 7.3), 3.90–4.10 (t, 2H, H-3, *J* = 7.3), 5.99–6.05 (d, 1H, H-3 furan), 6.30–6.37 (t, 1H, H-5 furan), 6.90–6.95 (s, 1H, vinyl-H), 7.10–7.50 (m, 6H, aromat+furan)
14	1605 (C=C)	2.43-2.57 (m, 2H, H-2), 2.96 (t, 2H, H-1, *J* = 7.3), 4.00 (t, 2H, H-3, *J* = 7.3), 6.02 (d,1H, H-3 furan), 6.34 (t, 1H, H-5 furan), 6.93 (s, 1H, vinyl-H), 7.10-7.50 (m, 6H, aromat+furan) 250/249/248 (23, 100, 16%, $M^{+\bullet}$), 221 (29%), 208 (9%, $C_{14}H_9NO$), 165 (9%, $C_{12}H_7N$)

4.2. 6-Heteroaryl-7-aryl-2,3-dihydropyrrolizines

The potency of anti-inflammatory drugs often depends especially on their lipophilicity. Replacement of a phenyl residue by a thiophene moiety significantly increases COX-1 inhibition [24].

Contrary to this hypothesis our 6-thiophene and 6-furane-7-aryl-2,3-dihydropyrrolizines possess no or poor COX-1/2 potency. The C-6 thiophene derivative **10** shows weak inhibition of COX-1/2 and 5-LOX at concentration of 10 μM (33, 50, 20%). The highest COX-1 inhibitory activity is found for the 3[1]-chlorothiophenederivative **11** (IC_{50}: 3.9 μM). Compound **12** inhibits the 5-LOX pathway noticeably (IC_{50}: 8.4 μM). In this series, **13** is the most potent COX-2 inhibitor (IC_{50}: 1.8 μM).

5. Summary and conclusions

These results indicate that the inhibitory effects of the compounds tested to inhibit COX-1/2 and 5-LOX can be shifted by varying R^1 and R^2:

a) Replacement of the phenyl-residue at C-6 by a thiophene or a furan moiety produces poor COX-1/2 or 5-LOX inhibitors.
b) The most potent COX-1 inhibitor (**8**) bears a methyl sulphide group at C-6. A lipophilic group at the C-7

residue increases COX-1 and especially COX-2 activity: **4** (26% inhibition at 0.1 μM) is ten times more potent than **1** (30% inhibition at 1 μM).

c) By oxidation of the sulphur atom, it is possible to shift the specificity for 5-LOX as well as for COX-2.

d) The combination of a methylsulphonyl group or methanesulphonamide R^1 with a lipophilic R^2 moiety produces triple inhibitors of COX-1/2 and 5-LOX (**2**, **3** and **9**). Compound **9** is a potent inhibitor of COX-1/2 and 5-LOX with a high selectivity to COX-2 in comparison to benzofuran derivatives and tebufelone with the same selectivity profile published. This combination of COX and 5-LOX inhibition with preference for COX-2 could offer opportunities to treat inflammatory diseases with better tolerated drugs, thus enhancing patient compliance. Investigations to confirm this hypothesis are ongoing.

6. Experimental

6.1. Chemistry

Melting points (m.p.) were determined on a Büchi SMP-20 apparatus and are uncorrected. All the compounds were analysed for C, H and N. Proton and ^{13}C-NMR were run on a Bruker AC-200 and AC-400 spectrometer using TMS as the internal standard. Mass spectra (EI) were obtained at 70 eV with a Varian MAT 7 spectrometer. IR spectra were obtained on a Perkin–Elmer 1310 spectrometer with KBr disks. Microanalyses were determined on a Haereus CHN rapid or a Carlo Erba Strumentazione1106. TLC plates of silica gel (Merck G_{254}) were used to monitor reaction development. Column liquid chromatography (silica gel 200–400 mesh, Merck) was used for product isolation from reaction mixtures; solvent A: toluol, solvent B: ethyl acetate–diisopropyl ether (8:2), solvent C: ethyl acetate–diisopropyl ether (6:4), solvent D: ether–dichloromethane (6:4), solvent E: ether, solvent F: PE–dichloromethane (1:1). All the reagents were of analytical grade and obtained as follows: salt for buffer solutions, solvents: Merck, Darmstadt (Germany); acetophenone derivatives **1**–**3**, **7**, calcium ionophore A 23187, diclofenac, indomethacin: Sigma, München (Germany), HPLC reference substances 12-HHT, internal standards PGB_2 and 15-keto-PGE_2: Paesel, Frankfurt/Main (Germany). Bovine blood was obtained from the local abattoir. Synthesis of 4″-Cl-SC 57666 was carried out according to reference [25].

6.1.1. General procedure for the preparation of 2,3 dihydro-1H-pyrrolizines (1–14)

2,3-Dihydro-1*H*-pyrrolizines were prepared according to reference [18].

6.2. Enzyme assays

For cell preparations and apparatus see references [19–22].

All the compounds were tested in an intact cell assay described earlier [20]. Shortly, the inhibition of COX-1 was determined using bovine thrombocytes and that of 5-LOX using bovine polymorphonuclear leucocytes (PMNLs). The cells were incubated with the compounds and stimulated with calcium ionophore A 23187. The amounts of LTB_4 and 5-HETE were quantified to measure 5-LOX activity. For the evaluation of the COX-1 12-HHT and PGE_2 were determined by HPLC.

This method does not give any information about the inhibition of COX-2, so the method of Bauer and Fiebich [21] was used to determine the inhibition of LPS-induced (10 ng ml^{-1}) COX-2 production of PGE_2 in human monocytes. The amounts of PGE_2 are determined by RIA. Another method to confirm the results, which is described earlier was used [22].

It allows the determination of IC_{50}-values for inhibitors of COX-1/2 on cells of the same origin: Unstimulated bovine aortic coronary endothelial cells (BAECs) were used as a source of COX-1 and BAECs pretreated with 100 μM acetylsalicylic acid (ASA) and activated with phorbol myristate acetate (PMA) were used as a source of COX-2. IC_{50} values were calculated with the program GRAFIT, Erithacus Software Ltd., UK. The standard deviations of the obtained values ($n = 4$) were less than 12% of the IC_{50} values of COX-1. Diclofenac, indomethacin and 4″-Cl-SC57666 were used as reference substances (Table 2).

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