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Original article

The pyrrole moiety as a template for COX-1/COX-2 inhibitors

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Abstract – Aroyl- and thiophene-substituted pyrrole derivatives have been synthesized as a new class of COX-1/COX-2 inhibitors. The inhibition of COX-1 was evaluated in a biological system using bovine PMNLs as the enzyme source, whereas LPS-stimulated human monocytes served as the enzyme source for inducible COX-2. The determination of the concentration of arachidonic acid metabolites was performed by HPLC for COX-1 and RIA for COX-2. Variation of the substitution pattern led to a series of active compounds which showed inhibition for COX-1 and COX-2. Structural requirements for the development of COX-1/COX-2 inhibitors are discussed. © 2000 Éditions scientifiques et médicales Elsevier SAS

COX-1/COX-2 inhibition / pyrrole derivatives / enzyme selectivity / structure–activity relationship

1. Introduction

NSAIDs are of huge therapeutic benefit in the treatment of inflammatory diseases. The most common side effects associated with all currently available NSAIDs are gastrointestinal haemorrhagia and ulceration [1]. These side effects during anti-inflammatory therapy are caused by interference with the physiological properties of prostaglandins.

The enzyme cyclooxygenase catalyses the addition of molecular oxygen to arachidonic acid to form the unstable PGG_2 which is then converted to the more stable PGH_2 by a peroxidase function. Two isoenzymes exist, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which differ in their regulation and tissue distribution.

COX-1 is constitutively expressed in cells 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). In contrast, COX-2 is a regulated enzyme that is induced by specific stimuli and is thought to be involved in inflammation and mitogenesis responses. Specific inhibitors of COX-2 would have the advantage of targeting the enzyme involved in the inflammatory processes and bypassing the constitutively expressed COX-1, i.e. eliminating unwanted side effects such as stomach ulceration while providing anti-inflammatory COX-2 inhibition.

Recently, it has been shown that COX-2 is constitutively expressed in the spinal cord [2], in the brain [3], in the kidney [4], in stomach mucosa [5] and in the pancreatic islet [6]. Although COX-2 inhibitors have anti-inflammatory properties, their greatest effects, according to recent clinical trial data, appear to be associated with pain relief and symptoms of osteoarthritis [7]. In human vessels COX-2 can produce beneficial mediators and possesses the same beneficial effects as COX-1, i.e. highly selective (> 1 000-fold) COX-2 inhibitors could have severe side effects, particularly in the cardiovascular system [8]. Therefore, it may be postulated that moderately selective COX-2 inhibitors may well represent a safer alternative in patients with underlying cardiovascular disease. There was no effect of celecoxib on platelet aggregation at all doses used. However, an inhibition of the urinary marker of systemic prostacyclin suggests that while platelet function is not inhibited,

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endothelial PG production may be reduced [9]. This may have implications for high risk cardiovascular patients, but there is no clinical experience yet to support this. There is also evidence that administration of COX-2 preferential and selective drugs produce fewer GIT side effects. However, once an ulcerative injury is present, COX-2 expression is elevated in response to this inflammation and the COX-2 enzyme seems to be essential for wound healing in the stomach by enhancing gastric blood flow, reducing gastric acid secretion and allowing epithelial cell proliferation and granulation tissue contraction [10, 11]. In consequence, highly selective COX-2 inhibitors can aggravate the injury and delay wound healing.

COX-2 is believed to make an important contribution to the increase in prostaglandins observed in pain and inflammation [12, 13]. Interestingly, a selective COX-1 inhibitor was an equipotent inhibitor of PG formation in an inflammatory exudate to the COX-2 selective inhibitor celecoxib but did not reduce increased PG levels in the cerebrospinal fluid, while non-selective COX-1/COX-2 inhibition appeared to be more effective in reducing signs of inflammation [13, 14].

These and other data suggest that the functions of COX-1 and COX-2 might be more complex than originally anticipated and that COX-1 inhibition might contribute to inhibition of prostaglandin production in inflammatory exudates. Therefore, a combined inhibition of COX-1 and COX-2 may result in a more efficient inhibition of chronic inflammation than a selective inhibition of COX-2.

There have been remarkable efforts concerning the identification of selective COX-2 inhibitors [15] with an attractive pharmacological profile; e.g. NS-398, DuP 697, SC-57666 and SC-558 (*figure 1*) have been reported as highly active COX-2-inhibitors [16–21]. Very recently the COX-2-selective inhibitors celecoxib [22, 23] and rofecoxib [24] (*figure 1*) have been pharmaceutically marketed for the indications of osteoarthritis, rheumatoid arthritis, acute pain in adults and dysmenorrhea, respectively.

Another interesting class of compounds to minimize the side effects 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.

Diarylpyrrolizines have been investigated as drugs acting on the COX-1, COX-2 and 5-LOX pathways of arachidonic acid metabolism. One potent compound of this new class is ML-3000 (*figure 2*) which may improve

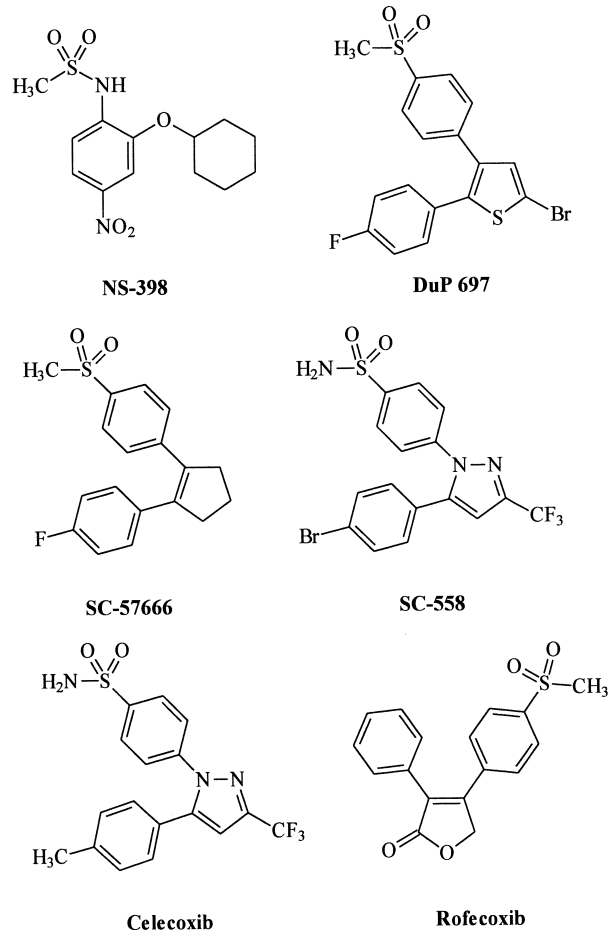


Figure 1. Structures of selective COX-2 inhibitors.

antirheumatic therapy by its pharmacodynamic and pharmacokinetic properties [25–27]. Recently 7-*tert*-butyl-2,3-dihydro-3,3-dimethylbenzofurane derivatives were reported as COX-2/5-LOX-inhibitors. One of these substances was found to be the active metabolite of tebufelone which acts as an antioxidant [28] (*figure 2*).

For meloxicam (*figure 2*) with IC_{50} values of 3.27 µM for COX-1 and 0.25 µM for COX-2, reduced side effects were reported according to the more selective COX-2 inhibition compared to COX-1 inhibition [29, 30].

COX-1/COX-2 inhibitors with similar inhibiting capacities – so called balanced or non-selective inhibitors (the term used depends on the scientific dogma the scientists are focusing on) – could improve the therapeutic benefit in the treatment of inflammatory diseases according to the phenomena discussed above.

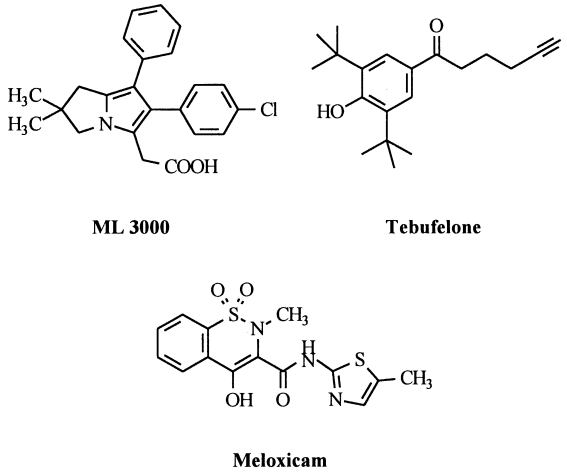


Figure 2. Structures of dual inhibitors (for explanations see text).

On our way to balanced COX-1/COX-2 inhibitors we intended to combine the structures of typical COX-2 inhibitors with the chemical requirements for COX-1 inhibition.

Altogether five structural classes of selective COX-2 inhibitors and a class bearing little or no resemblance to one another in their molecular structure have been identified [31]. In context with the known anti-inflammatory potency of keterolac [32] and tolmetin [33], characterized by an aroyl pyrrole moiety (*figure 3*), and the selective COX-2 inhibition of 1,2-diaryl pyrroles [20], we focused on 3-aroyl 4-aryl pyrrole derivatives inserting a carbonyl unit into the vicinal diaryl substitution which is the structural feature of a great member of selective COX-2 inhibitors. Thus, these structural modifications will verify the importance of vicinal aryl aroyl arrangements on potency and enzyme selectivity within the pyrrole series synthesized.

Ketorolac [32] and tolmetin [33] (*figure 3*) have meanwhile been withdrawn as systematically acting analgesics in Germany because of possible nephrotoxic risks [34, 35].

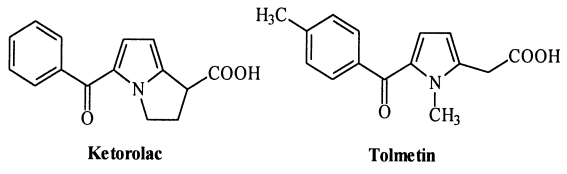


Figure 3. Structures of the pyrrole analgesics ketorolac and tolmetin.

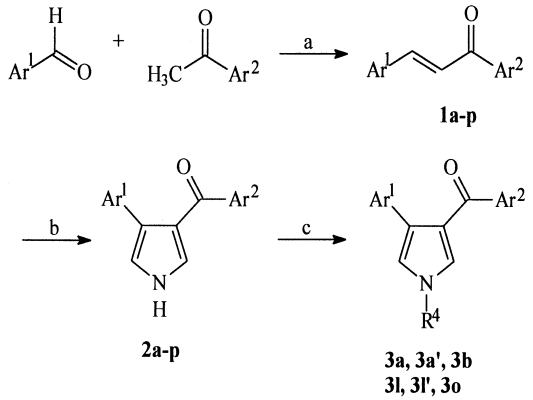


Figure 4. (a) KOH 15%, MeOH, 25 °C, 2 h, 63–93% yield. (b) TosMIC, NaH, THF, 25 °C, 0.5 h, 15–98% yield. (c) KOH, n-Bu_4NHSO_4, R_4I, CH_2Cl_2, 2 °C, 24 h, 45–96% yield; for Ar^1, Ar^2, R^4, see *table I*.

2. Chemistry

Chalcones as precursors for the chemical synthesis were appropriate tools for the synthesis of the desired aryl-aroyl-pyrrole derivatives. Chalcones themselves have been reported as substances with anti-inflammatory potency [36] on the one hand but cytotoxic effects on the other [37].

We followed the synthesis described by Artico and Di Santo [38, 39] starting from 1,3-diarylprop-2-en-1-ones with tosylmethyl isocyanide (TosMIC) to give 3-aroyl-4-arylpyrroles. TosMIC was used as a synthone for the pyrrole moiety which reacts with the corresponding chalcones in an intramolecular cyclization to the 3,4-disubstituted pyrroles. The ring carbons C(2) and C(5) neighbouring the nitrogen originate from TosMIC and C(3) and C(4) from the chalcones which act as Michael acceptors [40]. Variation at the substitution pattern was accomplished by using different kinds of chalcones (*figure 4*).

3. Biological assays

All the compounds were tested in an intact cell assay described earlier [41]. This assay was established to determine 5-LOX, 12-LOX and COX-1 activity. Bovine blood is used as the enzyme source and isolated platelets as the source for COX-1 activity. The cells were incubated with the compounds and stimulated with calcium ionophore A 23187. The amounts of LTB_4, 5-HETE, 12-HHT, PGE_2 and 12-HETE were determined by HPLC. IC_{50} values were calculated with the program GraFit, Erithacus Software Ltd., UK. The standard deviations of

Table I. Results of the in vitro assays with the aryl/aroyl-substituted pyrroles.

Compound	R^1	R^2	R^3	R^4	IC_{50} (μM) COX-1 [a]
2a	H	H	H	H	3.3
2b	H	H	Cl	H	4.8
2c	CH_3O	H	H	H	3.3
2d	F	H	H	H	1.5
2e	H	H	F	H	6.7
2f	H	CH_3O	H	H	10.0
2g	H	H	CH_3O	H	1.8
2h	CF_3	H	H	H	3.6
2i	H	H	CF_3	H	2.6
3a	H	H	H	CH_3	8.5
3a'	H	H	H	C_2H_5	9.8
3b	H	H	Cl	CH_3	10.0

[a] IC_{50} values represent the mean value of four determinations.

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.

This method does not give any information about the inhibition of COX-2. Meanwhile different systems to measure COX-2 activity are available; for example Patrignani [42] describes a method quantifying PGE_2 production in plasma from LPS-stimulated human whole blood using a specific RIA. We used the method of Bauer and Fiebich [43] where the inhibition of LPS-induced (10 ng/ml) COX-2 production of PGE_2 in human monocytes is determined. This in vitro model uses monocytes which are collected from the peripheral blood of healthy donors with a cell number of 106 cells/well on a microtitre plate. The amounts of PGE_2 are determined by RIA.

4. Results and discussion

Tables I and *II* show the compounds synthesized by the general procedure (*figure 4*). A differentiation can be made between N-unsubstituted aryl-aroyl-pyrroles, thienoyl-aryl-pyrroles or thienyl-aroyl-pyrroles and the corresponding N-substituted compounds. At first, all the compounds were tested for their ability to inhibit COX-1. The most potent compounds underwent further investigation to ascertain their COX-2 inhibiting potency.

4.1. Aryl-aroyl-pyrroles

Table I summarizes the impact of additional substituents both on the aryl and aroyl moiety. The compounds inhibit the COX pathway significantly. Alkylation at the pyrrole nitrogen reduces the inhibitory potency. Compounds **3a**, **3a'** and **3b** are less active COX-1 inhibitors compared to the corresponding unsubstituted **2a** and **2b**. We chose **2a** with an IC_{50} value of 3.3 μM for COX-1 as a lead structure for further modifications in order to increase COX-1 and COX-2 inhibition. As shown in *table II* introduction of thienyl residues increased the potencies remarkably.

4.2. Pyrroles with a thienyl/thienoyl moiety

The potency of anti-inflammatory drugs often depends on their lipophilicity, especially, replacement of a phenyl residue by a thiophene moiety significantly increases COX-1 inhibition [44]. Looking for balanced COX-1/COX-2 inhibitors we modified **2a** systematically by thiophene substitution to obtain more potent compounds (*table II*). Compared to **2a** the thienyl derivatives **2k** and **2m** are 2- and 3-fold more active inhibitors of COX-1.

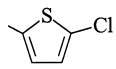
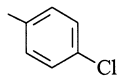
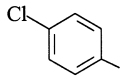
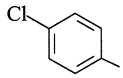
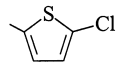
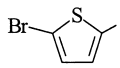
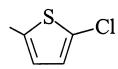
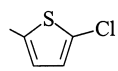
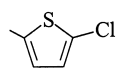
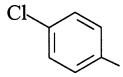
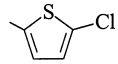
Table II gives further information about structure–activity relationships improving COX-1 inhibition. Compound **2k** compared to **2m** (1.45 vs. 1.15 μM) and **2j** compared to **2n** (1.2 vs. 0.37 μM) proves that chlorination of the phenyl moiety amplifies the activity, probably due to increased lipophilicity. The same phenomenon is seen with the exchange of a thiophene (**2j**) by a chlorothiophene moiety (**2l**) (1.2 vs. 0.18 μM) due to increased lipophilicity. The most potent compound in this series with an IC_{50} value of 0.07 μM for COX-1 is **2o** with a chlorophenyl and a chlorothienyl moiety.

The most potent COX-inhibitors (**2j**–**2p**) underwent COX-2 screening in the model mentioned. *Figure 5* proves that the increase of COX-1 inhibition is correlated with a proportional improvement of COX-2 inhibition. According to the IC_{50} values for COX-2, **2l**, **2m**, **2n** and **2o** are characterized as balanced inhibitors of both COX-1 and COX-2 (IC_{50} ratios between 0.26 and 1.15). As shown in tables *I* and *II*, N-alkylation in general decreases the inhibitory potency (**3l**, **3l'** and **3o**).

Clearly, an unsubstituted pyrrole-nitrogen atom together with the thiophene nucleus represents a requirement for optimal COX-1/COX-2 inhibition. It is not easy to explain COX-1 and COX-2 inhibitory effects by structural differentiation of the compounds. Especially, the isoform COX-2 seems to tolerate a lot of different structural parameters, as reflected by the great variety of COX-2 pharmacophores [31]. The aspect of lipophilicity is important for COX-1 and COX-2 inhibition as seen by thiophene and chlorothiophene substitution.

Figure 6 gives the IC_{50} values for diclofenac, indomethacin and 4''-Cl-SC 57666 and leads to the question: what makes cyclopentene derivative 4''-Cl-SC 57666

Table II. Results of the in vitro assays with **2a** and the thiophene substituted pyrroles.

Compound	R^1	R^2	R^3	IC_{50} (μM) [a] COX-1	COX-2	COX-1/ COX-2 ratio
2a			H	3.30	6.00	0.55
2j			H	1.20	16% (10 μM)	n.d.
2k			H	1.45	5.00	0.29
2l			H	0.18	0.35	0.51
2m			H	1.15	1.00	1.15
2n			H	0.37	0.95	0.39
2o			H	0.07	0.27	0.26
2p			H	0.10	0.70	0.14
3l			CH_3	3.90	27% (10 μM)	n.d.
3l'			C_2H_5	2.20	n.t.	n.d.
3o			C_2H_5	33% (10 μM)	n.t.	n.d.
diclofenac				0.0028	0.0004	7
indomethacin				0.004	0.0005	8
4''-Cl-SC 57666				> 10	0.016	> 625

n.t.: not tested;. n.d.: not determined.. [a] IC_{50} values represent the mean values of four determinations and were calculated by regression analysis.

[45] a highly selective COX-2 inhibitor compared to diclofenac and indomethacin?

The interaction of drugs with the protein structures of COX-1 [46] or COX-2 [47] is an appropriate tool for elucidating pharmacological action. The structure of bovine COX-1 complexed with several NSAIDs as well as the structure of unliganded murine COX-2 and the corresponding complexes with flurbiprofen, indometha-

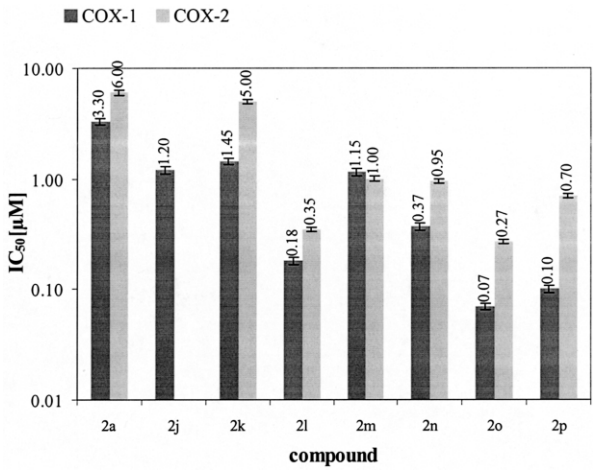


Figure 5. Graphic comparison of IC_{50} values (COX-1 and COX-2) of the pyrrole derivatives.

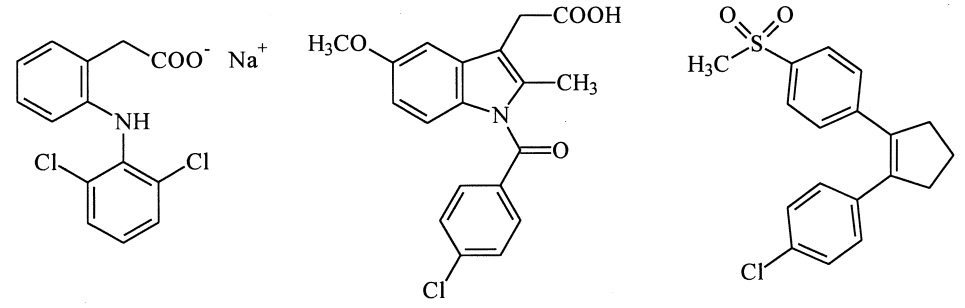
cin and the selective COX-2 inhibitor SC-558 have been published [46, 47]. The time-dependent inhibition of COX-2 as a consequence of conformational changes is based on this information.

Consistant with a high sequence identity (approximately 60%) the overall structures of COX-1 and COX-2 are highly conserved. The significant difference between the two enzymes seems to be the much larger binding site in COX-2 for NSAIDs.

This binding site seems to be a pocket caused by the substitution of a valine (COX-2) for an isoleucine (COX-1) at position 523 at the active site of the cyclooxygenase which allows access to the additional pocket, whereas access to this 'side pocket' is restricted in COX-1 because of the bulkier isoleucine. Hence, COX-2 offers an additional pocket beside the main channel which is responsible for the selectivity [46]. A further exchange valine/isoleucine at position 434 of the amino acid chain leads to the formation of a 'gate'. This gate is closed in COX-1 because of the larger isoleucine side chain. In COX-2 with the smaller side chain at position 434, the gate has room to swing open allowing the entrance of more bulky compounds such as 4''-Cl-SC-57666, a selective COX-2 inhibitor [45] and modified indomethacin derivatives [48].

The selectivity of 4''-Cl-SC-57666 seems to result from the phenylsulphone moiety which binds in a pocket that is more restricted in COX-1 and is unoccupied in complexes of COX-2 with non-selective inhibitors. 4''-Cl-SC-57666, a weak competitive COX-1 inhibitor, inhibits COX-2 in a slow, time-dependent process.

Indomethacin causes a slow, time-dependent inhibition of COX-1 and COX-2. The time-dependence may result from the formation of a salt bridge between the carboxylic function of the drug and Arg 120 of the enzyme. Indomethacin, which initiates conformational changes of the enzyme, binds deeply within the cyclooxygenase binding site and penetrates furthest into the hydrophobic channel, but not deeply enough to enhance COX-2 selectivity. The benzoyl moiety seems to play another important role for the COX-1 activity of indomethacin. It can be deduced that the benzoyl group enhances COX-1



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

Figure 6. Chemical structures, IC_{50} values for COX-1 and COX-2 and the corresponding COX-1/COX-2 ratios of diclofenac, indomethacin and cyclopentene derivative 4''-Cl-SC-57666.

inhibition because derivatives with a benzyl instead of a benzoyl group do not show COX-1 but COX-2 selectivity [48].

In addition, COX-1 inhibitors such as indomethacin lose their COX-1 potency if bulkier substituents at the heterocyclic nucleus prevent an efficient binding to the restricted COX-1 pocket.

Compounds **2l**, **2m**, **2n** and **2o** combine the structural requirements for both COX-1 and COX-2 inhibition. The keto group attached to the pyrrole nucleus might be a reason for the COX-1 activity being similar to indomethacin where the benzoyl residue at the nitrogen atom enhances the affinity for COX-1.

Furthermore, it is possible that the NH-function of these pyrrole derivatives leads to H-bridges between the nitrogen of the ligand and the protein structure of the enzyme. Analogous to indomethacin it can be supposed that the 'selectivity pocket' of COX-2 is not completely occupied by **2l**, **2m**, **2n** and **2o**, i.e. these compounds are less potent COX-2 inhibitors than the reference 4''-Cl-SC-57666.

5. Conclusions

Our results indicate that the compounds tested represent a new template for anti-inflammatory drugs. It is evident that the combination of structural elements which are so far known for COX-1 and COX-2 inhibition leads to active compounds which show a balanced inhibition of the COX-isoenzymes and offers the opportunity to treat inflammatory diseases with better tolerated drugs, thus enhancing patient compliance. Further investigations to refine structural parameters for an optimum efficacy and to study the tolerance of the drugs are ongoing.

6. Experimental protocols

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. Proton and ^{13}C-NMR were run on 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 an Heraeus CHN rapid or a Carlo Erba Strumentazione 1106 and were within ± 0.4% of theoretical values. TLC plates of silica gel (Merck G_{254}) were used to monitor reaction development and to check purity of all the compounds. Column liquid chromatography (silica gel 200–400 mesh, Merck) was used for product isolation from reaction mixtures.

All reagents were of analytical grade and obtained as follows: salt for buffer solutions, solvents: Merck, Darmstadt (Germany); chalcone derivatives **1a–c** and **1g**, 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 ref. [45].

6.1. Chemistry

6.1.1. General procedure for the preparation of compounds **1d**–**f** *and* **1h**–**p**

The mixture of the aromatic aldehyde (10 mmol) and the acetylated aromate (10 mmol) were dissolved in 30 mL of MeOH and stirred during addition of 5 mL of potassium hydroxide (15% m/V). After some minutes the product precipitated. About 2 mL of glacial acetic acid were added. Solids were then filtered with suction and washed twice with cold MeOH. The chalcones formed in this manner were directly used for the preparation of the corresponding pyrroles without any further purification.

6.1.1.1. (E)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one **1d** *[49]*

4-Fluorobenzaldehyde (50 mmol, 6.21 g), acetophenone (50 mmol, 6.01 g), KOH-soln. approx. 2 mL; 89% yield (10.1 g) as yellow crystals, m.p. 84–86 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) ν_{Ar-H} 3 050, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 580, 1 500, ν_{C-F} 1 200; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 6.6–8.0 (m, 11H, aromatic and vinylic H); MS *m/z* (rel. int.) = 226 (100%, M^+), 149 (49%, $M^+–C_6H_5$), 130 (18%, $M^+–C_6H_5–F$); calcd. for $C_{15}H_{11}FO$ = 226.08 g/mol.

6.1.1.2. (E)-1-(4-Fluorophenyl)-3-phenylprop-2-en-1-one **1e** *[50]*

Benzaldehyde (20 mmol, 2.14 g), 4-fluoroacetophenone (20 mmol, 2.76 g), KOH-soln. approx. 1 mL, 71% (3.2 g) yield as yellow crystals, m.p. 73–75 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) ν_{Ar-H} 3 050, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 580, 1 500, ν_{C-F} 1 200; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 7.3–8.3 (m, 11H, aromatic and vinylic H); MS *m/z* (rel. int.) = 226 (100%, M^+), 131 (34%, $M^+–C_6H_5–F$), 103 (37%, $C_6H_5–CH=CH$); calcd. for $C_{15}H_{11}FO$ = 226.08 g/mol.

6.1.1.3. (E)-1-(4-Chlorophenyl)-3-(2-methoxy-phenyl)prop-2-en-1-one **1f**

2-Methoxybenzaldehyde (30 mmol, 4.08 g), 4-chloroacetophenone (30 mmol, 4.64 g), KOH-soln. approx. 1.5 mL, 85% yield (6.96 g) as yellow powder, m.p.

66–68 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 090, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 580, 1 500; MS *m/z* (rel. int.) = 272 (9%, $M^+\cdot$), 241 (100%, $M^+–OCH_3$), 139 (12%, $Cl–C_6H_4–CO$); calcd. for $C_{16}H_{13}ClO_2$ = 272.55 g/mol.

6.1.1.4. (E)-1-Phenyl-3-(4-trifluoromethylphenyl)prop-2-en-1-one **1h** *[51]*

4-Trifluoromethylbenzaldehyde (30 mmol, 5.22 g), acetophenone (30 mmol, 4.95 g), KOH-soln. approx. 1.5 mL, 68% yield (5.68 g) as yellow crystals, m.p. 116–118 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 090, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 570, $\nu_{C–F}$ 1 200; MS *m/z* (rel. int.) = 276 (87%, $M^+\cdot$), 207 (25%, $M^+–CF_3$), 199 (21%, $M^+–C_6H_5$), 103 (50%, $C_6H_5–CH=CH$); calcd. for $C_{16}H_{11}F_3O$ = 276.08 g/mol.

6.1.1.5. (E)-1-(4-Trifluoromethylphenyl)-3-phenylprop-2-en-1-one **1i** *[52]*

Benzaldehyde (25 mmol, 2.40 g), 4-trifluoromethylacetophenone (25 mmol, 3.76 g), KOH-soln. approx. 1.5 mL, 63% yield (3.46 g) as white–yellow crystals, m.p. 108–111 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 100, $\nu_{C=O}$ 1 660, $\nu_{Ar\ C=C}$ 1 570, 1 450, $\nu_{C–F}$ 1 220; MS *m/z* (rel. int.) = 276 (100%, $M^+\cdot$), 145 (23%, $F_3C–C_6H_4$), 103 (28%, $C_6H_5–CH=CH$); calcd. for $C_{16}H_{11}F_3O$ = 276.08 g/mol.

6.1.1.6. (E)-3-Phenyl-1-(thien-2-yl)prop-2-en-1-one **1j** *[53]*

Benzaldehyde (30 mmol, 3.21 g), 2-acetylthiophene (30 mmol, 3.79 g), KOH-soln. approx. 1.5 mL, 92% yield (5.91 g) as yellow powder, m.p. 77–79 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 090, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 570, 1 450; MS *m/z* (rel. int.) = 213 (100%, $M^+–1$), 185 (20%); calcd. for $C_{13}H_{10}OS$ = 214.05 g/mol.

6.1.1.7. (E)-3-(Thien-2-yl)-1-phenylprop-2-en-1-one **1k** *[54]*

Thiophene-2-carbaldehyde (50 mmol, 5.61 g), acetophenone (50 mmol, 6.0 g), KOH-soln. approx. 2 mL, 89% yield (9.48 g) as yellow powder, m.p. 67–69 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 090, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 560, 1 470; MS *m/z* (rel. int.) = 214 (99%, $M^+\cdot$), 137 (57%, $M^+–C_6H_5$), 109 (41%, $M^+–C_6H_5–CO$); calcd. for $C_{13}H_{10}OS$ = 214.05 g/mol.

6.1.1.8. (E)-1-(5-Chlorothien-2-yl)-3-phenylprop-2-en-1-one **1l** *[55]*

Benzaldehyde (60 mmol, 6.42 g), 2-acetyl-5-chlorothiophene (60 mmol, 9.63 g), KOH-soln. approx. 3 mL, 89% yield (13.48 g) as yellow powder, m.p. 91–93 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 100, $\nu_{C=O}$ 1 640, $\nu_{Ar\ C=C}$ 1 580, 1 520; MS *m/z* (rel. int.) = 248 (100%, $M^+\cdot$), 213 (24%, $M^+–Cl$), 145 (53%, $M^+–C_6H_5–CH=CH$), 103 (68%, $C_6H_5–CH=CH$); calcd. for $C_{13}H_9ClOS$ = 248.49 g/mol.

6.1.1.9. (E)-1-(4-Chlorophenyl)-3-(thien-2-yl)- prop-2-en-1-one **1m** *[56]*

Thiophene-2-carbaldehyde (10 mmol, 1.12 g), 4-chloroacetophenone (10 mmol, 1.54 g), KOH-soln. approx. 2 mL, 75% yield (1.86 g) as yellow powder, m.p. 111–113 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 100, $\nu_{C=O}$ 1 640, $\nu_{Ar\ C=C}$ 1 570, 1 540, $\nu_{C–Cl}$ 1 000; MS *m/z* (rel. int.) = 248 (100%, $M^+\cdot$), 213 (24%, $M^+–Cl$), 139 (47%, $M^+–C_6H_5–CH=CH$), 109 (71%, $C_6H_5–CH=CH$); calcd. for $C_{13}H_9ClOS$ = 248.49 g/mol.

6.1.1.10. (E)-3-(4-Chlorophenyl)-1-(thien-2-yl)-prop-2-en-1-one **1n** *[53]*

2-Acetylthiophene (15 mmol, 2.10 g), 4-chlorobenzaldehyde (15 mmol, 1.89 g), KOH-soln. approx. 3 mL, 75% yield (2.82 g) as white–yellow crystals, m.p. 132–133 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 080, $\nu_{C=O}$ 1 640, $\nu_{Ar\ C=C}$ 1 570, 1 540, $\nu_{C–Cl}$ 1 000; MS *m/z* (rel. int.) = 247 (100%, $M^+\cdot–1$), 212 (57%, $M^+\cdot–Cl$), 196 (36%), 110 (68%); calcd. for $C_{13}H_9ClOS$ = 248.49 g/mol.

6.1.1.11. (E)-3-(4-Chlorophenyl)-1-(5-chloro-thien-2-yl)-prop-2-en-1-one **1o** *[56]*

2-Acetyl-5-chlorothiophene (20 mmol, 3.21 g), 4-chlorobenzaldehyde (20 mmol, 2.81 g), KOH-soln. approx. 3 mL, 93% yield (5.29 g) as white–yellow crystals, m.p. 132–133 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 080, $\nu_{C=O}$ 1 650, $\nu_{Ar\ C=C}$ 1 570, 1 540, $\nu_{C–Cl}$ 1 010; MS *m/z* (rel. int.) = 281 (100%, $M^+\cdot–1$), 246 (95%, $M^+–Cl$), 164 (37%, $M^+–C_4H_2S–Cl$), 144 (72%, $CO–C_4H_2S–Cl$); calcd. for $C_{13}H_8Cl_2OS$ = 282.94 g/mol.

6.1.1.12. (E)-3-(5-Bromothien-2-yl)-1-(5-chloro-thien-2-yl)-prop-2-en-1-one **1p**

2-Acetyl-5-chlorothiophene (24.5 mmol, 3.95 g), 5-bromothiophene-2-carbaldehyde (24.5 mmol, 4.68 g), KOH-soln. approx. 3 mL, 69% yield (5.65 g) as dark yellow powder, m.p. 139–140 °C (MeOH); IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar–H}$ 3 080, $\nu_{C=O}$ 1 640, $\nu_{Ar\ C=C}$ 1 580, 1 540, $\nu_{C–Cl}$ 1 020; MS *m/z* (rel. int.) = 334 (11%, $M^+\cdot$), 253 (100%, $M^+–Br$), 145 (72%, $CO–C_4H_2S–Cl$); calcd. for $C_{11}H_6BrClOS_2$ = 333.34 g/mol.

6.1.2. General procedure for preparation of the pyrroles **2a–2p**

To a stirred suspension of sodium hydride in abs. THF under nitrogen, the solution of the 1:1 mixture of TosMIC and the corresponding chalcone was added so slowly that the hydrogen formation could be easily controlled. After

finishing addition, the mixture was stirred for another 15 min, then concentrated in vacuo and quenched with 100 mL of water. The aqueous phase was acidified with 1 N sodium hydroxide solution, extracted three times with ethyl acetate. Combined organic phases were dried over sodium sulphate, concentrated in vacuo and the residue was treated with cold MeOH to obtain the product which was recrystallized using cold ethylacetate.

6.1.2.1. 3-Benzoyl-4-phenyl-1H-pyrrole **2a** *[56]*

1,3-Diphenylprop-2-en-1-one (**1a**, 60 mmol, 16.66 g), TosMIC (60 mmol, 11.71 g), sodium hydride (72 mmol, 1.73 g), 91% yield (13.53 g) as white powder, m.p. 229–231 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 180, ν_{Ar-H} 2 980, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 570, 1 550; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.1–7.8 (m, 12H, H arom), 11.7 (s, 1H, NH); MS *m/z* (rel. int.) = 247 (68%, $M^+\cdot$), 170 (100%, $M^+–C_6H_5$). Anal. $C_{17}H_{13}NO$ (C, H, N).

6.1.2.2. 3-(4-Chlorbenzoyl)-4-phenyl-1H-pyrrole **2b**

(*E*)-1-(4-Chlorphenyl)-3-phenylprop-2-en-1-one (**1b**, 60 mmol, 14.49 g) TosMIC (60 mmol, 11.71 g), sodium hydride (72 mmol, 1.73 g), 95% yield (16.05 g) as white powder, m.p. 219–221 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 180, ν_{Ar-H} 2 980, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 570, 1 550, ν_{C-Cl} 1 020; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.4 (m, 7H, H arom), 7.4–7.5 (d, 2H, *J* = 8.1 Hz, H-3', H-5'), 7.6–7.7 (d, 2H, *J* = 8.2 Hz, H-2', H-6'), 11.7 (s, 1H, N*H*); MS *m/z* (rel. int.) = 281 (67%, $M^+\cdot$), 170 (100%, $M^+–C_6H_5$), 115 (42%). Anal. $C_{17}H_{12}ClNO$ (C, H, N).

6.1.2.3. 3-Benzoyl-(4-methoxyphenyl)-1H-pyrrole **2c** *[56]*

(*E*)-3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (**1c**, 25 mmol, 5.95 g), TosMIC (25 mmol, 4.88 g), sodium hydride (30 mmol, 0.72 g), 59% yield (4.10 g) as white powder, m.p. 218–220 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 190, ν_{Ar-H} 2 990, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 580, 1 550; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 3.7 (s, 3H, CH_3O), 6.7–7.8 (m, 11H, H arom), 11.7 (s, 1H, NH); MS *m/z* (rel. int.) = 277 (100%, $M^+\cdot$), 219 (13%). Anal. $C_{18}H_{15}NO_2$ (C, H, N).

6.1.2.4. 3-Benzoyl-4-(4-fluorophenyl)-1H-pyrrole **2d**

(*E*)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (**1d**, 40 mmol, 9.04 g), TosMIC (40 mmol, 7.81 g), sodium hydride (48 mmol, 1.15 g), 98% yield (10.35 g) as white crystals, m.p. 204–206 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 180, ν_{Ar-H} 2 980, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 570, 1 550, ν_{C-F} 1 210; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.8 (m, 11H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 265 (88%, $M^+\cdot$), 188 (100%, $M^+–C_6H_5$). Anal. $C_{17}H_{12}FNO$ (C, H, N).

6.1.2.5. 3-(4-Fluorobenzoyl)-4-phenyl-1H-pyrrole **2e**

(*E*)-1-(4-Fluorophenyl)-3-phenylprop-2-en-1-one (**1e**, 14 mmol, 3.17 g), TosMIC (14 mmol, 2.73 g), sodium hydride (17 mmol, 0.40 g), 93% yield (3.47 g) as white crystals, m.p. 217–219 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 180, ν_{Ar-H} 2 980, 2 950, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 590, 1 550, ν_{C-F} 1 210; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.8 (m, 11H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 265 (85%, $M^+\cdot$), 170 (100%, $C_6H_4–CO–C_4H_3N$), 115 (40%). Anal. $C_{17}H_{12}FNO$ (C, H, N).

6.1.2.6. 3-(4-Chlorobenzoyl)-4-(2-methoxyphenyl)-1H-pyrrole **2f**

(*E*)-1-(4-Chlorophenyl)-3-(2-methoxyphenyl)-prop-2-en-1-one (**1f**, 25 mmol, 6.81 g), TosMIC (25 mmol, 4.88 g), sodium hydride (30 mmol, 0.72 g), 71% yield (5.49 g) as white powder, m.p. 219–221 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 200, ν_{Ar-H} 2 970, 2 950, $\nu_{C=O}$ 1 620, $\nu_{Ar\ C=C}$ 1 570, ν_{C-Cl} 1 010; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 3.7 (s, 3H, CH_3O), 7.0–7.8 (m, 11H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 311 (72%, $M^+\cdot$), 280 (110%, $M^+–CH_3O$), 185 (28%), 171 (68%, $C_6H_4–CO–C_4H_3N$). Anal. $C_{18}H_{14}ClNO_2$ (C, H, N).

6.1.2.7. 3-(4-Methoxybenzoyl)-4-phenyl-1H-pyrrole **2g**

(*E*)-1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-one (**1g**, 25 mmol, 5.96 g), TosMIC (25 mmol, 4.88 g), sodium hydride (30 mmol, 0.72 g), 15% yield (1.03 g) as white powder, m.p. > 180 °C (decomp., ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 200, ν_{Ar-H} 2 980, 2 950, $\nu_{C=O}$ 1 620, $\nu_{Ar\ C=C}$ 1 590, 1 500; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 3.7 (s, 3H, CH_3O), 7.0–7.8 (m, 11H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 277 (17%, $M^+\cdot$), 262 (26%, $M^+–CH_3$), 184 (40%), 135 (75%, $CO–C_6H_4–OCH_3$), 84 (100%). Anal. $C_{18}H_{15}NO_2$ (C, H, N).

6.1.2.8. 3-Benzoyl-4-(4-trifluoromethylphenyl)-1H-pyrrole **2h**

(*E*)-1-Phenyl-3-(4-trifluoromethylphenyl)prop-2-en-1-one (**1h**, 10 mmol, 2.76 g), TosMIC (10 mmol, 1.95 g), sodium hydride (12 mmol, 0.29 g), 74% yield (2.33 g) as white powder, m.p. 249–251 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) ν_{N-H} 3 170, ν_{Ar-H} 2 980, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 570, 1 550, ν_{C-F} 1 210; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.1–8.0 (m, 11H, H arom), 11.8 (s, 1H, NH);

MS *m/z* (rel. int.) = 315 (100%, $M^+\cdot$), 238 (89%, $M^+–C_6H_5$), 141 (70%, $C_6H_5–C_4H_3N$). Anal. $C_{18}H_{12}F_3NO$ (C, H, N).

6.1.2.9. 4-Phenyl-3-(4-trifluoromethylbenzoyl)-1H-pyrrole **2i**

(*E*)-1-(4-Trifluoromethylphenyl)-3-phenylprop-2-en-1-one (**1i**, 10 mmol, 2.76 g), TosMIC (10 mmol, 1.95 g), sodium hydride (12 mmol, 0.29 g), 52% yield (1.64 g) as white powder, m.p. 215–217 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 180, $\nu_{Ar–H}$ 2 980, $\nu_{C=O}$ 1 600, $\nu_{Ar\ C=C}$ 1 560, 1 500, $\nu_{C–F}$ 1 210; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.1–8.0 (m, 11H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 315 (6%, $M^+\cdot$), 290 (14%), 231 (13%), 139 (32%), 100 (100%). Anal. $C_{18}H_{12}F_3NO$ (C, H, N).

6.1.2.10. 3-Phenyl-4-(thien-2-oyl)-1H-pyrrole **2j** *[56]*

(*E*)-3-Phenyl-1-(thien-2-yl)prop-2-en-1-one (**1j**, 30 mmol, 5.57 g), TosMIC (30 mmol, 5.08 g), sodium hydride (36 mmol, 0.86 g), 66% yield (4.34 g) as white–yellow powder, m.p. 168–171 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 190, $\nu_{Ar–H}$ 2 980, $\nu_{C=O}$ 1 630, $\nu_{Ar\ C=C}$ 1 580, 1 510; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.09 (t, 1H, *J* = 2.5 Hz, H-4''), 7.15–7.20 (m, 2H, H-2'', H-6''), 7.23–7.29 (t, 2H, *J* = 7.5 Hz, H-3'', H-5''), 7.33–7.38 (d, 2H, *J* = 6.7 Hz, H-5, H-5'), 7.52 (t, 1H, *J* = 2.5 Hz, H-2), 7.72 (d, 1H, *J* = 4.2 Hz, H-4'), 7.92 (d, 1H, *J* = 4.6 Hz, H-3'), 11.7 (s, 1H, NH); MS *m/z* (rel. int.) = 253 (100%, $M^+\cdot$),220 (69%), 170 (44%, $M^+–C_4H_3S$), 115 (44%). Anal. $C_{15}H_{11}NOS$ (C, H, N).

6.1.2.11. 3-Benzoyl-4-(thien-2-yl)-1H-pyrrole **2k**

(*E*)-3-(Thien-2-yl)-1-phenylprop-2-en-1-one (**1k**, 43 mmol, 9.20 g), TosMIC (43 mmol, 8.40 g), sodium hydride (52 mmol, 1.25 g), 73% yield (7.94 g) as white–yellow powder, m.p. 224–226 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 160, $\nu_{Ar–H}$ 2 970, $\nu_{C=O}$ 1 590, $\nu_{Ar\ C=C}$ 1 590, 1 550; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.8 (m, 10H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 253 (80%, $M^+\cdot$), 176 (100%, $M^+–C_6H_5$). Anal. $C_{15}H_{11}NOS$ (C, H, N).

6.1.2.12. 3-(5-Chlorothien-2-oyl)-4-phenyl-1H-pyrrole **2l** *[56]*

(*E*)-1-(5-Chlorothien-2-yl)-3-phenylprop-2-en-1-one (**1l**, 10 mmol, 2.49 g), TosMIC (10 mmol, 1.95 g), sodium hydride (12 mmol, 0.29 g), 42% yield (1.20 g) as white powder, m.p. 188–191 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 300, $\nu_{Ar–H}$ 2 990, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 590, 1 510, $\nu_{C–Cl}$ 1 020; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.8 (m, 10H, H arom), 11.8 (s, 1H, NH); MS *m/z*(rel. int.) = 287 (19%, $M^+\cdot$), 252 (10%, M^+–Cl), 170 (18%, $C_6H_5–CO–C_4H_2N$), 115 (100%). Anal. $C_{15}H_{10}ClNOS$ (C, H, N).

6.1.2.13. 3-(4-Chlorobenzoyl)-4-(thien-2-yl)-1H-pyrrole **2m**

(*E*)-1-(4-Chlorobenzoyl)-3-(thien-2-yl)-prop-2-en-1-one (**1m**, 10 mmol, 2.49 g), TosMIC (10 mmol, 1.95 g), sodium hydride (12 mmol, 0.29 g), 70% yield (2.0 g) as yellow crystals, m.p. 191-193 °C (ethyl acetate); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 300, $\nu_{Ar–H}$ 2 990, 2 940, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 590, 1 510, $\nu_{C–Cl}$ 1 020; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 6.95–7.0 (t, 1H, *J* = 4.1 Hz, H-4thiophene), 7.2–7.35 (m, 4H, H arom), 7.5–7.55 (d, 2H, *J* = 8 Hz), 7.7–7.75 (d, 2H, *J* = 8.3 Hz), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 287 (87%, $M^+\cdot$), 176 (100%, $M^+–C_4H_3S–CO$). Anal. $C_{15}H_{10}ClNOS$ (C, H, N).

6.1.2.14. 3-(4-Chlorophenyl)-4-(thien-2-oyl)-1H-pyrrole **2n**

(*E*)-3-(4-Chlorophenyl)-1-(thien-2-yl)-prop-2-en-1-one (**1n**, 10 mmol, 2.48 g), TosMIC (10 mmol, 1.95 g), sodium hydride (13 mmol, 0.31 g), 97% yield (2.81 g) as white crystals, m.p. 184–185 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 180, $\nu_{Ar–H}$ 2 990, 2 940, $\nu_{C=O}$ 1 620, $\nu_{Ar\ C=C}$ 1 590, 1 510, $\nu_{C–Cl}$ 1 020; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.1–7.9 (m, 9H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 286 (10%, $M^+\cdot$), 155 (51%), 91 (100%). Anal. $C_{15}H_{10}ClNOS$ (C, H, N).

6.1.2.15. 3-(4-Chlorophenyl)-4-(5-chlorothien-2-oyl)-1H-pyrrole **2o**

(*E*)-3-(4-Chlorophenyl)-1-(5-chlorothien-2-yl)-prop-2-en-1-one (**1o**, 13 mmol, 3.68 g), TosMIC (13 mmol, 2.54 g) sodium hydride (17 mmol, 0.40 g), 81% yield (3.40 g) as white–yellow powder, m.p. 229–230 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) $\nu_{N–H}$ 3 200, $\nu_{Ar–H}$ 2 990, 2 940, $\nu_{C=O}$ 1 600, $\nu_{Ar\ C=C}$ 1 580, 1 520, $\nu_{C–Cl}$ 1 030; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.1–7.6 (m, 8H, H arom), 11.8 (s, 1H, NH); MS *m/z* (rel. int.) = 320 (100%, $M^+\cdot$-1), 285 (60%, M^+–Cl), 203 (62%, $M^+–C_4H_2$–Cl). Anal. $C_{15}H_9Cl_2NOS$ (C, H, N).

6.1.2.16. 3-(5-Bromothien-2-yl)-4-(5-chlorothien-2-oyl)-1H-pyrrole **2p**

(*E*)-3-(5-Bromothien-2-yl)-1-(5-chlorothien-2-yl)-prop-2-en-1-one (**1p**, 15 mmol, 5.0 g), TosMIC (15 mmol, 2.93 g), sodium hydride (20 mmol, 0.48 g), 59% yield (3.29 g) as dark-yellow fine crystals, m.p. 156–157 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) $\nu_{C–H}$ 3 220, $\nu_{Ar–H}$ 2 990, 2 940, $\nu_{C=O}$ 1 600, $\nu_{Ar\ C=C}$ 1 580, 1 520, $\nu_{C–Br}$ 1 130, $\nu_{C–Cl}$ 1 030; ^{1}H-NMR (200 MHz, DMSO-d_6, ppm) δ 7.0–7.6 (m, 6H, H arom), 11.9 (s, 1H, NH);

MS *m/z* (rel. int.) = 372 (100%, $M^+\cdot$), 292 (59%, M^+–Br), 145 (35%, Cl–C_4H_2S–CO). Anal. $C_{13}H_7BrClNOS_2$ (C, H, N).

6.1.3. General procedure for N-alkylation of the pyrroles

The pyrrole was suspended in 40 mL dichloromethane. Powdered potassium hydroxide and a catalytic amount of tetra-n-butylammonium hydrogensulphate were added. The mixture was sonicated for 2 min at room temperature and then cooled to 0 °C. Alkyl iodide dissolved in dichloromethane was added rapidly, the ice bath removed and the mixture stirred overnight. After that 50 mL of water were added, the aqueous layer extracted exhaustively with dichloromethane, the combined organic layers dried over sodium sulphate, concentrated under reduced pressure and the residue purified by column chromatography on silica using dichloromethane as eluent.

6.1.3.1. 3-Benzoyl-1-methyl-4-phenyl-1H-pyrrole **3a**

3-Benzoyl-4-phenyl-*1H*-pyrrole (**2a**, 10 mmol, 2.47 g) potassium hydroxide (20 mmol, 1.12 g), methyl iodide (20 mmol, 2.84 g), 45% yield (1.18 g) as white powder, m.p. 109–111 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 3 140, ν_{Ar-H} 2 990, 2 940, $\nu_{C=O}$ 1 630, $\nu_{Ar\ C=C}$ 1 600, 1 550; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 3.7 (s, 3H, CH_3), 6.7–7.8 (m, 12H, H arom); MS *m/z* (rel. int.) = 261 (78%, $M^+\cdot$), 184 (100%, M^+–C_6H_5). Anal. $C_{18}H_{15}NO$ (C, H, N).

6.1.3.2. 3-Benzoyl-1-ethyl-4-phenyl-1H-pyrrole **3a'**

3-Benzoyl-4-phenyl-*1H*-pyrrole (**2a**, 5 mmol, 1.24 g), potassium hydroxide (10 mmol, 0.56 g), ethyl iodide (10 mmol, 1.56 g), 46% yield (0.63 g) as white powder, m.p. 104–107 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 3 040, ν_{Ar-H} 2 970, $\nu_{C=O}$ 1 630, $\nu_{Ar\ C=C}$ 1 590, 1 540; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 1.5 (t, 3H, *J* = 7.3 Hz, CH_3), 3.9 (quart, 2H, *J* = 7.3 Hz, CH_2), 6.8–7.8 (m, 12H, H arom); MS *m/z* (rel. int.) = 275 (68%, $M^+\cdot$), 198 (100%, M^+–C_6H_5), 115 (24%). Anal. $C_{19}H_{17}NO$ (C, H, N).

6.1.3.3. 3-Chlorobenzoyl-1-methyl-4-phenyl-1H-pyrrole **3b**

3-(4-Chlorobenzoyl)-4-phenyl-*1H*-pyrrole (**2b**, 10 mmol, 2.82 g), potassium hydroxide (20 mmol, 1.12 g), methyl iodide (20 mmol, 2.84 g), 94% yield (2.80 g) as white powder, m.p. 218–221 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 3 020, ν_{Ar-H} 2 940, $\nu_{C=O}$ 1 620, $\nu_{Ar\ C=C}$ 1 570, 1 540, $\nu_{C=Cl}$ 1 030; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 3.7 (s, 3H, CH_3), 7.0–7.4 (m, 7H, H arom), 7.5–7.8 (2d, 4H, *J* = 8.2 Hz); MS *m/z* (rel. int.) = 295 (49%, $M^+\cdot$), 184 (100%, M^+–Cl–C_6H_4), 115 (24%). Anal. $C_{18}H_{15}ClNO$ (C, H, N).

6.1.3.4. 3-(5-Chlorthien-2-oyl)-1-methyl-4-phenyl-1H-pyrrole **3l** *[56]*

3-(5-Chlorothien-2-oyl)-4-phenyl-*1H*-pyrrole (**2l**, 4 mmol, 1.15 g), potassium hydroxide (8 mmol, 0.45 g), methyl iodide (8 mmol, 1.14 g), 74% yield (0.89 g) as light yellow powder, m.p. 123–125 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 3 110–3 060, ν_{Ar-H} 2 950–2 900, $\nu_{C=O}$ 1 600, $\nu_{Ar\ C=C}$ 1 550, 1 510, $\nu_{C=Cl}$ 1 030; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 3.7 (s, 3H, CH_3), 7.0–7.6 (m, 9H, H arom); MS *m/z* (rel. int.) = 300 (16%, $M^+\cdot$–1), 225 (13%, M^+–C_6H_5), 218 (12%), 145 (21%, CO–C_4H_2S–Cl), 130 (27%), 68 (100%). Anal. $C_{16}H_{12}ClNOS$ (C, H, N).

6.1.3.5. 3-(5-Chlorothien-2-oyl)-1-ethyl-4-phenyl-1H-pyrrole **3l'**

3-(5-Chlorothien-2-oyl)-4-phenyl-*1H*-pyrrole (**2l**, 8 mmol, 2.30 g), potassium hydroxide (16 mmol, 0.90 g), ethyl iodide (16 mmol, 2.49 g), 93% yield (2.36 g) as light yellow powder, m.p. 84–87 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 3 100–3 020, ν_{Ar-H} 2 960, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 550, 1 510, $\nu_{C=Cl}$ 1 030; ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 1.4 (t, 3H, *J* = 7.3 Hz, CH_3), 4.0 (quart, 2H, *J* = 7.3 Hz, CH_2), 7.1–7.4 (m, 7H, H arom), 7.6–7.7 (m, 2H, H arom); MS *m/z* (rel. int.) = 315 (19%, $M^+\cdot$), 269 (14%), 198 (21%), 155 (20%), 112 (52%, C_4H_4S–CO), 84 (46%, C_4H_4S), 69 (74%), 56 (100%). Anal. $C_{17}H_{14}ClNOS$ (C, H, N).

6.1.3.6. 3-(4-Chlorophenyl)-4-(5-chlorothien-2-oyl)-1-ethyl-1H-pyrrole **3o**

3-(4-Chlorophenyl)-4-(5-chlorothien-2-oyl)-*1H*-pyrrole (**2o**, 3.5 mmol, 1.13 g), ethyl iodide (8.75 mmol, 1.36 g), potassium hydroxide (10.5 mmol, 0.59 g), 96% yield (1.18 g) as yellow powder, m.p. 89–92 °C (diisopropyl ether); IR ν_{max} (KBr, cm^{-1}) ν_{C-H} 2 950, ν_{Ar-H} 2 940, $\nu_{C=O}$ 1 610, $\nu_{Ar\ C=C}$ 1 560, 1 520, $\nu_{C=Cl}$ 1 030; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 1.5 (t, 3H, *J* = 7.3 Hz, CH_3), 4.0 (quart, 2H, *J* = 7.3 Hz, CH_2), 6.75–6.85 (2d, 2H, *J* = 3.5 Hz, $H_{thiophene}$), 7.2–7.4 (m, 6H, H arom); MS *m/z* (rel. int.) = 350 (78%, $M^+\cdot$), 315 (65%, M^+–Cl), 284 (20%, M^+–Cl–C_2H_5), 231 (100%), 196 (50%). Anal. $C_{17}H_{13}Cl_2NOS$ (C, H, N).

6.2. Enzyme assays

For cell preparations and apparatus see refs. [41] and [43].

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