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Bioorganic & Medicinal Chemistry 14 (2006) 918–927
Bioorganic & Medicinal Chemistry

Synthesis, molecular modelling and enzymatic evaluation of (±)3,5-diphenyl-2-thioxoimidazolidin-4-ones as new potential cyclooxygenase inhibitors

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Received 26 April 2005; revised 1 September 2005; accepted 6 September 2005
Available online 7 October 2005

Abstract—A series of substituted (±)3,5-diphenyl-2-thioxoimidazolin-4-ones was synthesized in order to design new type-2 cyclooxygenase (COX-2) inhibitors. This study has led to molecules which completely inhibit human recombinant COX-2 at 50 μM. Molecular modelling highlighted drug interactions with the active site of both cyclooxygenases and suggested modifications to enhance the selectivity of the compounds. In human blood, COX-2 expression was then induced by LPS, and the inhibitory potency of these drugs was disappointing. This weak activity was attributed to a poor aqueous stability of these imidazolidinones substituted by two aryl in position 3 and 5 (15 min < $t_{1/2}$ < 130 min). The improvement of the stability of this heterocycle could generate a novel template to treat COX-associated diseases such as arthritis, rheumatoid polyarthritis and cancer.

1. Introduction

Besides lipoxygenases and epoxygenases, type-1 and type-2 cyclooxygenases (COX-1 and COX-2) are considered as the starting point of the metabolism of arachidonic acid, the precursor of prostaglandins (PGs) and thromboxane (TX).[1] Contrary to COX-1, which is constitutively expressed in many organs and tissues, COX-2 expression is induced in several cell types by lipopolysaccharide (LPS),[2] cytokines (IL-1, TNFα, etc.),[3] hormones (FSH, LH),[4] growth factors (EGF, PDGF, FGF)[5,6] or oncogenes (v-Src, vRas).[7] Initially, the design of selective COX-2 inhibitors began with the aim to develop anti-inflammatory drugs (NSAIDs) with reduced side effects at gastric and renal levels. More recently, COX-2 overexpression has been demonstrated in several types of cancer,[8,9] in angiogenesis[10,11] and in neurodegenerative diseases such as Alzheimer's[12] or Parkinson's.[13] From a structural point of view, selective COX-2 inhibitors are divided into five classes[14,15]: (i) ethers or thioethers (nimesulide, NS-398, flosulide, L-745337), (ii) vicinal diaryl carbocycles or heterocycles (coxibs, SC-57666, etc.), (iii) structurally modified NSAIDs (APHS, L-748780, etc.), (iv) antioxidants (S-2474, etc.) and (v) olefins (*cis*-stilbenes, triaryl-*Z*-alkenes). The marketed coxibs are characterized by a 1,2-diarylheterocycle. Generally, the heterocycle is a five-membered ring such as thiophene (DuP-697),[16] furanone (rofecoxib),[17] pyrazole (celecoxib),[18] oxazole (JTE-522),[19] isoxazole (valdecoxib, parecoxib),[20,21] thiazole,[22] imidazole,[23,24] pyrrole,[25] or oxazolone.[26] Some coxibs such as etoricoxib[27] have a six-membered ring as central heterocycle (pyranone, pyridine and pyridazinone).[28,29] Recently, rofecoxib was voluntarily withdrawn from the market because of an increased risk of cardiovascular adverse events with a probability linked to the dose and the duration of treatment.[30,31] For targeting COX-2 isoform, it is therefore interesting to design new molecule scaffolds different from 1,2-diaryl heterocyclic type derivatives such as rofecoxib. In this work, we report the synthesis, the COX inhibitory potency and docking studies of (±)2-thioxoimidazolidin-4-ones bearing two substituted aryl moieties in position 3 and 5.

Keywords: Ovine COX-1; Human COX-2; COX inhibition; 2-Thioxoimidazolin-4-ones.
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doi:10.1016/j.bmc.2005.09.005

2. Chemistry

The (±)3,5-diaryl-2-thioxoimidazolidin-4-ones were prepared by the cyclisation of a thiourea **4** formed by reaction of D,L- α-phenylglycine **3** with the required isothiocyanate **2**[32,33] (Fig. 1). According to this pathway, the aryl in position 3 is the moiety of the corresponding isothiocyanate, whereas the phenyl present in position 5 is the side chain of D,L- α-phenylglycine. 4-Methylsulfonyl, 3-methylsulfonyl and 4-aminosulfonyl isothiocyanates are commercially unavailable. These aryl isothiocyanates were prepared from the corresponding primary amine **1** with *N*,*N*′-thiocarbonyldiimidazole (TCDI)[34] (Fig. 1). A series of (±)3,5-diphenyl-2-thioxoimidazolidin-4-ones was then prepared (**5**–**23**) (Table 1). A wide variety of substituents were placed on the 3-aryl residue including the aminosulfonyl present in the structures of celecoxib and valdecoxib and the methylsulfonyl present in the position para of the aryl rings of rofecoxib and etoricoxib. Each compound was obtained as a racemate since none of them exhibited optical rotation ($c = 5$, $CHCl_3$).

3. Results and discussion

3.1. Cyclooxygenases inhibition

Each compound reported here was assayed for inhibition of ovine COX-1 (*o*COX-1) and human recombinant COX-2 (*h*COX-2). The inhibitory potency of each molecule (50 μM) is expressed as the decrease of $PGF_{2\alpha}$ obtained by chemical reduction of PGH_2 produced by COXs using arachidonic acid as substrate (Table 1). Except for the iodo (**11**) and the aminosulfonyl derivatives (**15**), the introduction of a substituent in *para* position of the phenyl (**6**–**15**) increases the *h*COX-2 inhibitory potency. For the *meta*-substituted molecules (**16**–**23**), a similar trend is observed, except for the 3-methyl (**16**), the 3-chloro (**19**) and the 3-iodo (**21**) derivatives which are as or less active on *h*COX-2 than their unsubstituted parent **5**. Within the halo-substituted compounds, the fluoro (**8**, **18**) and bromo (**10**, **20**) derivatives are the most active on *h*COX-2 whatever their position on the phenyl ring (*meta* or *para*). The methylsulfonyl moiety (**14**, **23**) increases the inhibitory potency on *h*COX-2 particularly when placed in *meta*-position (**23**). Both CF_3-substituted compounds (**12**, **22**) are among the most active compounds on *h*COX-2.

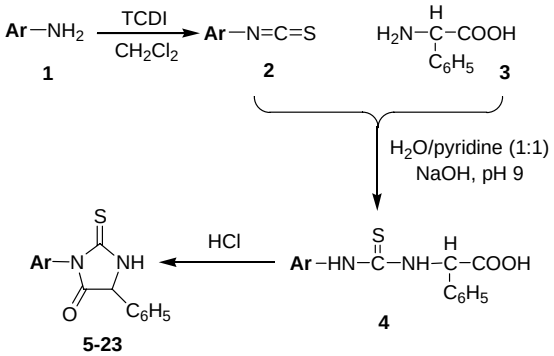

Figure 1. Synthetic pathway of 3,5-diphenyl-2-thioxoimidazolidin-4-ones.

Table 1. Structure of 3,5-diphenyl-2-thioxoimidazolidin-4-ones and inhibitory potency on isolated *o*COX-1 and recombinant *h*COX-2

Compound 50 μM	Ar	Inhibitory potency (%)	
		*o*COX-1	*h*COX-2
5	Phenyl	67 ± 13	63 ± 4
6	4-CH_3-phenyl	75 ± 8	89 ± 1
7	4-C_2H_5-phenyl	8 ± 4	81 ± 9
8	4-F-phenyl	91 ± 3	88 ± 5
9	4-Cl-phenyl	57 ± 6	70 ± 9
10	4-Br-phenyl	76 ± 4	84 ± 10
11	4-I-phenyl	40 ± 6	60 ± 9
12	4-CF_3-phenyl	67 ± 2	98 ± 1
13	4-NO_2-phenyl	12 ± 2	68 ± 5
14	4-CH_3SO_2-phenyl	61 ± 9	74 ± 9
15	4-NH_2SO_2-phenyl	41 ± 7	42 ± 12
16	3-CH_3-phenyl	33 ± 9	60 ± 8
17	3-CH_3O-phenyl	48 ± 7	85 ± 5
18	3-F-phenyl	72 ± 6	88 ± 5
19	3-Cl-phenyl	20 ± 9	59 ± 3
20	3-Br-phenyl	48 ± 5	93 ± 3
21	3-I-phenyl	31 ± 7	48 ± 6
22	3-CF_3-phenyl	44 ± 9	85 ± 6
23	3-CH_3SO_2-phenyl	38 ± 7	90 ± 3
Celecoxib		29 ± 6	98 ± 2

When compared to **5**, the introduction of a substituent in *meta* position (**16**–**23**) reduces the inhibitory potency on *o*COX-1, except for the fluoro derivative (**18**) which is also the most active on *o*COX-1 in the *para* series (**8**). In the *para* series, the methyl (**6**) and bromo (**10**) compounds are more active on *o*COX-1 than their parent compound (**5**). As expected, celecoxib, chosen as COX-2 selective inhibitor, was more potent on *h*COX-2 than on *o*COX-1. At 50 μM, celecoxib completely inhibited *h*COX-2 and is less potent on *o*COX-1.

3.2. Molecular modelling

Whatever the isozyme concerned, the COX binding site can be considered as a hydrophobic channel extending from the membrane binding domain to the core of the catalytic domain. In the upper part of the channel, both isozymes possess a Ser_{530} which is the amino acid acetylated by aspirin, whereas Tyr_{385} located at the top of the channel is involved in the hydroperoxidase activity. Two charged residues, Arg_{120} and Glu_{524}, are also present in the COX active site of both isozymes. The main difference between the two COX active sites is the replacement of two Ile (Ile_{434} and Ile_{523}) in COX-1 by two less bulky amino acids (Val_{434} and Val_{523}) in COX-2.[35] This double substitution opens a polar side-pocket, enlarging the volume of the COX-2 active site and giving access to Arg_{513} replaced in COX-1 by a histidine.

Second, in the apex of the COX-2 binding site, the substitution of Phe_{503} in COX-1 by Leu_{503} generates a small alcove which is hydrophobic due to the presence of

Leu_{384}, Tyr_{385} and Trp_{387} (Fig. 2). The binding modes of compounds **12**, **22** and **23** in the ovine crystallized COX-1 (PDB entry: 1EQH) and human modelled COX-2 were predicted by docking studies. The two isomers of each compound were docked using three algorithms (Research, Gold and Autodock).[36–38] The solutions common to the three programs assumed to be the most probable were then refined with Discover3 module[39] in order to account for amino acid flexibility of the active site. In this paper, only the compounds with the *S* configuration are described but the same conclusion can be made for the *R* isomer.

The binding mode, observed for **12** and **22** in the COX-2 binding site, called 'alcove mode' is quite similar in both isoforms. In COX-2, the compounds lie in the hydrophobic channel and do not fill the polar side-pocket (Fig. 3a). Particularly, the CF_3 moiety of **12** and **22** fills the apex of the channel and particularly the lipophilic alcove. This group is H-bounded to the nitrogen of the indole ring of Trp_{387}.

In COX-1, **12** and **22** are also restrained in the channel but due to steric hindrance between the CF_3 group and Leu_{384}, interactions with the enzyme are less favourable (data not shown).

The binding mode of the methylsulfone **23** with the COX-2 isoform, the 'lateral mode', is quite different from that observed for the CF_3-derivatives **12** and **22**

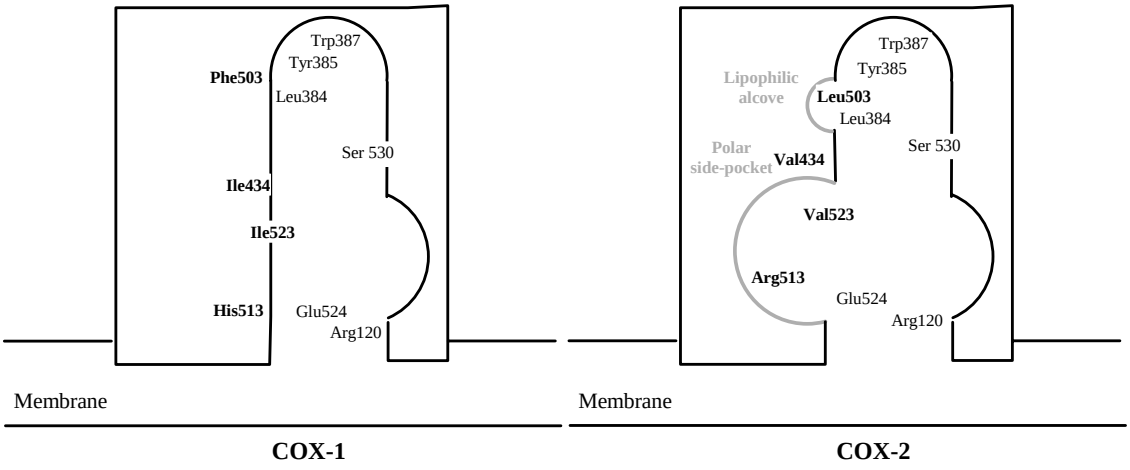

Figure 2. Schematic representation of the active site of the two isoenzymes COX-1 and COX-2.

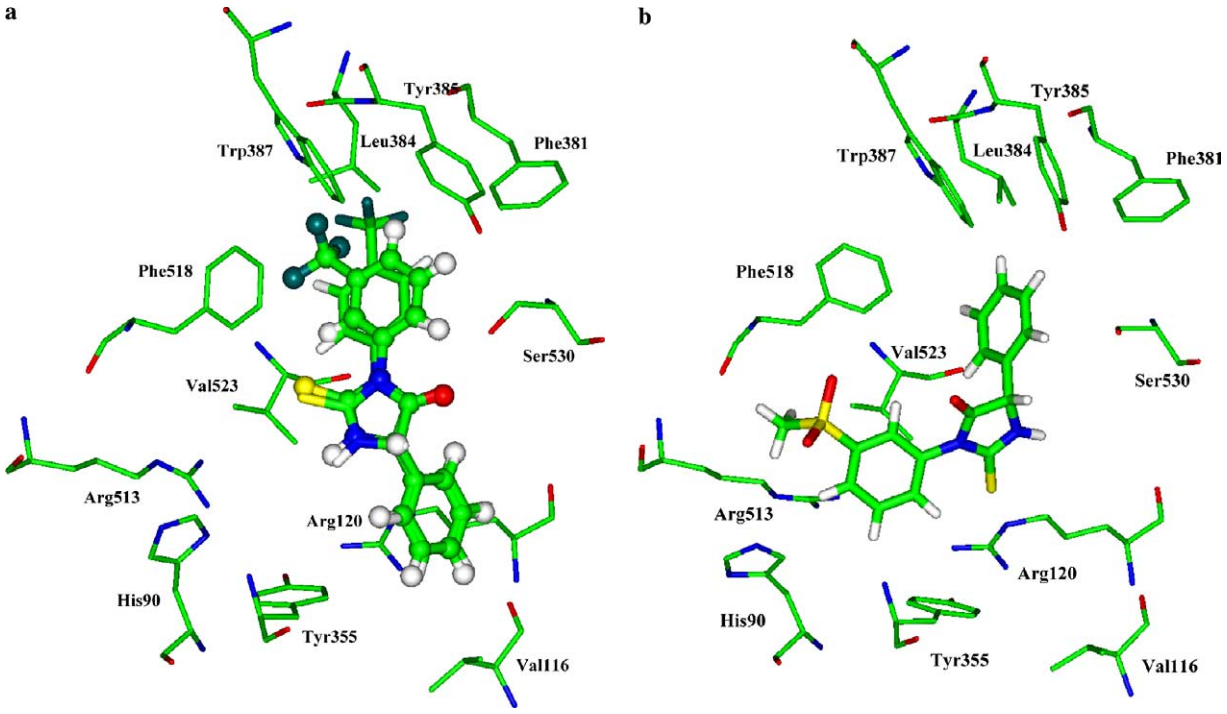

Figure 3. (a) 'Alcove mode' of **12** (stick) and **22** (ball and stick) in the active site of the human COX-2. (b) 'Lateral mode' of **23** in the active site of the human COX-2.

(Fig. 3b). Indeed, the 3-CH_3SO_2-phenyl moiety, in COX-2, fills into the polar side-pocket and is H-bounded to Phe_{518} and Gln_{192}. A CH···π interaction is also observed between Phe_{518} and the 5-phenyl group which lies in the upper part of the channel. In COX-1, on the other hand, **23** is not more able to fit the hydrophilic pocket and the sulfone moiety is located in the top of the active site (data not shown). Unfavourable interactions are therefore observed in the COX-1 isoform besides the COX-2 one. The polar side-pocket, specific to COX-2, would be therefore responsible for the high COX-2 inhibitory potency of **23**, like nimesulide[40] and SC-558, an analogue of celecoxib.[41] On the other hand, the high *h*COX-2 inhibitory potency of **12** and **22** (Table 1) would be due to the filling of the lipophilic alcove only present in this isoform. Compound **20**, one of the most potent inhibitors of the series, bearing also a large hydrophobic substituent, would bind in the same way. In both COX-2 binding modes, the 2-thioxoimidazolidin-4-ones are not able to fit all the volume of the active site. In the 'alcove' and 'lateral mode', the polar side-pocket and the bottom of the channel are not filled, respectively.

In order to enhance COX-2 selectivity and activity of this type of compounds, it would be therefore interesting to add a polar (like a ketobenzyl) or a hydrophobic substituent (like a benzyl) in position 1 of the 2-thioxoimidazolidin-4-one ring as exemplified in Figure 4. The proposed molecules have a larger molecular volume (332 $Å^3$ for structure a; 321 $Å^3$ for structure b) than **12** (225 $Å^3$), **22** (225 $Å^3$) and **23** (244 $Å^3$). They would therefore better fit the COX-2 active site.

3.3. Inhibition of human blood cyclooxygenases

At 50 μM, the most potent COX-2 inhibitors of this series are the 4-CF_3-phenyl derivative (**12**, 98% *h*COX-2 inhibition), the 3-Br-phenyl compound (**20**, 93% *h*COX-2 inhibition) and the 3-methylsulfophenyl (**23**, 90% *h*COX-2 inhibition) as active as celecoxib (98% *h*COX-2 inhibition). These four molecules have been selected to evaluate their cyclooxygenase inhibitory potency in the human whole blood assay. The unsubstituted diphenyl compound **5** chosen as parent compound has also been included for further evaluation.

In this human whole blood test, the COX-1 activity is expressed as the amount of TXB_2, the stable metabolite of TXA_2, produced by human platelets activated by incubation with calcimycin for 10 min. As compared to their inhibitory potency measured on isolated COX-1 isozyme, the inhibitory activity of compounds **5** and **12** strongly decreases from 67% to 5% and 24%, respectively, as observed for **23** (38–2%) (Table 2). At 50 μM in the whole blood assay, celecoxib reinforces its COX-1 inhibitory potency, while the activity of **20** is only reduced from 48% to 31%. COX-2 expression in human monocytes and prostaglandin production has been induced by blood incubation with lipopolysaccharide (100 μg mL^{-1}) for 24 h. In these conditions, COX-2 activity is expressed, and its activity determined as the PGE_2 production. In this blood assay, compounds **5** and **12** are inactive (Table 2).

When compared to its inhibitory potency on isolated *h*COX-2, the activity of 2-thioxoimidazolidin-4-ones **20** and **23** drops from 93% and 90% to 47% and 13%, respectively. As previously demonstrated,[42] celecoxib (50 μM) completely inhibits COX-2 and COX-1 isoforms in human blood cells.

Table 2. Inhibitory potency of COX-1 and COX-2 isozymes in human whole blood and half-life of selected 2-thioxoimidazolidin-4-ones

Compound (50 μM)	Inhibitory potency (%)		Half-life (min)
	COX-1	COX-2	
5	5 ± 4.1	7 ± 5.3	29.3
12	24 ± 6.2	6 ± 3.8	30.2
20	31 ± 5.0	47 ± 5.2	130.5
23	2 ± 3.5	13 ± 5.1	14.2
Celecoxib	100 ± 3.9	98 ± 0.1	

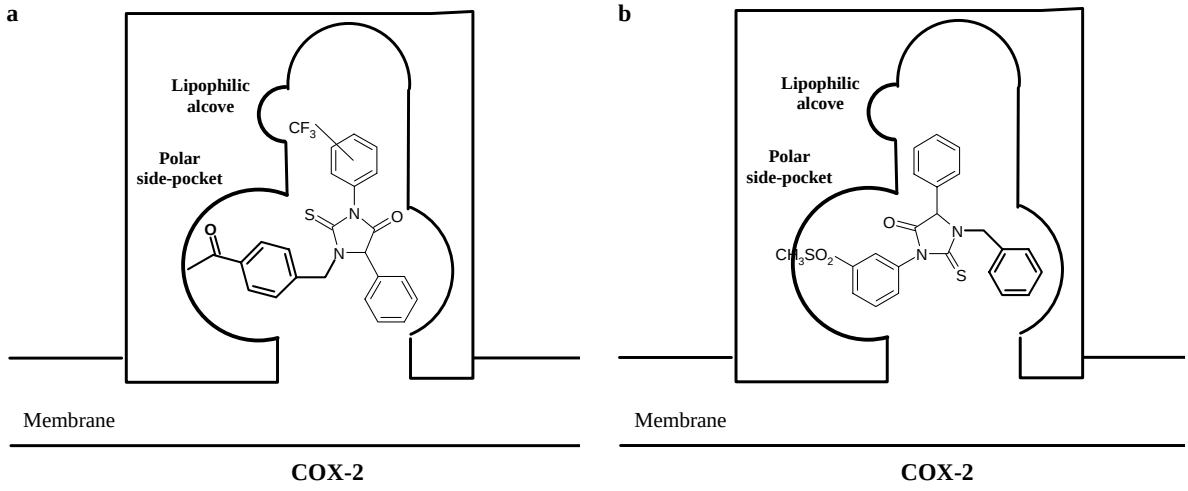

Figure 4. Proposed structural modifications for: (a) **12** and **22**; (b) **23**.

Among the parameters explaining these disappointing results observed for the 2-thioxoimidazolidin-4-ones, the drug stability has been pointed out and investigated by LC/MS. In water, the half-life ($t_{1/2}$) of compounds **5** and **12** was only of 30 min. The less stable compound was **23** ($t_{1/2}$ = 14 min), whereas **20** exhibited a higher stability with a half-life higher than 2 h (Table 2). The very short half-life of **5**, **12** and **23** could explain the strong drop of activity observed in the blood test, particularly for their COX-2 inhibitory potency. Indeed, when the activity of the potential inhibitors is evaluated on isolated recombinant isozyme, the investigated drug is only incubated for 5 min with the enzyme and 2 min more with arachidonic acid used as substrate. In the whole blood assay used to determine the inhibitory potency on COX-1, the drug is incubated for 15 min with blood, then 10 min more in the presence of the platelet activator prior to stopping the reaction and dosing TXB_2. In the case of COX-2, the drug is incubated for 24 h in the presence of lipopolysaccharide before PGE_2 quantification. This long incubation period coupled to a very short half-life of **5**, **12** and **23** could explain their inefficacy on COX-2. Due to a longer half-life, the COX-2 inhibitory potency of **20** is partly preserved.

4. Conclusion

IIn this work, we present the development of a new series of thioxoimidazolidinones as potential new inhibitors of type-2 cyclooxygenase. Contrary to coxibs, which are mainly 1,2-diarylheterocycles, this new series is characterized by a 3,5-diaryl substitution. The new synthesized compounds have been tested on isolated enzyme (*o*COX-1 and *h*COX-2) and several compounds strongly (**20** and **23**) or completely (**12**) inhibit recombinant *h*COX-2 at 50 μM. In order to optimize and to enhance the selectivity over COX-2, we carried out a molecular modelling study. This study suggests the incorporation of a polar or hydrophobic substituent in position 1 of the 2-thioxoimidazolidin-4-ones in order to better fill the COX-2 active site. The efficacy of the most active compounds was also evaluated on a human whole blood test. Unfortunately, on this test, their potency strongly decreased. In contrast to celecoxib, these disappointing results have been attributed to the poor stability of these (±)3,5-diphenyl-2-thioxoimidazolidin-4-ones. The improvement of the stability of this heterocycle could generate a novel template to treat COX-associated diseases such as arthritis, rheumatoid polyarthritis and cancer.

5. Experimental

5.1. Chemistry

Melting points were determined with a Büchi-Tottoli B540 apparatus in open capillary tubes and are uncorrected. IR spectra were recorded as KBr pellets on a Bio-Rad FTS-165 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were taken on a Jeol JNMEX-400 (400 MHz) instrument in DMSO-d_6 with TMS as internal standard at 20 °C; chemical shifts are reported in δ values (ppm) relative to internal TMS (singlet, δ = 0 ppm). For ^{1}H NMR spectra, the abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, and m = multiplet are used throughout. Elemental analyses (C, H, N and S in %) were performed with a Thermo Finnigan Flash EA 1112-elemental analyzer.

5.1.1. Synthesis of aryl isothiocyanates (2)

5.1.1.1. 4-Methylsulfonyl isothiocyanate. Triethylamine (319 μL, 2.29 mmol) was added to 4-methylsulfonylaniline hydrochloride (500 mg, 2.29 mmol) suspended in CH_2Cl_2 (5 mL) for 30 min. *N*,*N*′-thiocarbonyldiimidazole (420 mg, 2.29 mmol) is dissolved in CH_2Cl_2 (5 mL) and cooled to 5 °C. This solution is added dropwise to the solution containing 4-methylsulfonylaniline. The reactive mixture is allowed to reach room temperature (25 °C) and is stirred for 12 h. The solvents are evaporated under reduced pressure, and the crude residue is purified by column chromatography using silica as stationary phase and cyclohexane/ethyl acetate (1:1) as mobile phase. Yield: 74%. Mp: 134 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.27 (s, 3H, CH_3), 7.70 (d, 2H, 2H- and 6H-Ph, J = 8.80 Hz), 8.00 (d, 2H, 3H- and 5H-Ph, J = 8.80 Hz). ^{13}C NMR (DMSO-d_6) δ ppm: 43.35 (CH_3), 127.02 (2C, C3C5-Ph), 128.94 (2C, C2C6-Ph), 136.51 (C1-Ph), 139.55 (C4-Ph), 179.70 (NCS). IR (KBr): 3088 (ν ArCH), 3022 (ν ArCH), 2926 (ν CH), 2194–2121 (ν N=C=S), 1585 (ν C–C), 1305–1284 (ν_{as} SO_2), 1144 (ν_s SO_2) cm^{-1}. Anal. Calcd for $C_8H_7NO_2S_2$: C, 45.05; H, 3.31; N, 6.57; S, 30.07. Found: C, 44.84; H, 3.10; N, 6.64; S, 30.21.

5.1.1.2. 3-Methylsulfonyl isothiocyanate. The title compound is synthesized according to the procedure described for 4-methylsulfonyl isothiocyanate, except for the reaction time which was only 3.5 h. This synthesis was performed with 3-methylsulfonylaniline hydrochloride (1 g, 4.57 mmol) and *N*,*N*′-thiocarbonyldiimidazole (840 mg, 4.57 mmol). Yield: 72%. Mp: 80–81 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.29 (s, 3H, CH_3), 7.71–7.80 (m, 2H, 5H- and 6H-Ph), 7.91 (dt, 1H, 4H-Ph, J = 7.60 and 1.60 Hz), 8.01 (t, 1H, 2H-Ph, J = 1.80 Hz). ^{13}C NMR (DMSO-d_6) δ ppm: 43.57 (CH_3), 124.79 (C2-Ph), 126.03 (C4-Ph), 129.80 (C5-Ph), 130.90 (C5-Ph), 131.26 (C6-Ph), 140.29 (C3-Ph), 180.31 (CS). IR (KBr): 3072 (ν ArCH), 3016 (ν ArCH), 2941 (ν CH), 2073 (ν N=C=S), 1588 (ν C–C), 1314–1294 (ν_{as} SO_2), 1136 (ν_s SO_2) cm^{-1}. Anal. Calcd for $C_8H_7NO_2S_2$: C, 45.05; H, 3.31; N, 6.57; S, 30.07. Found: C, 44.86; H, 3.02; N, 6.67; S, 30.26.

5.1.1.3. 4-Aminosulfonyl isothiocyanate. *N*,*N*′-thiocarbonyldiimidazole (2.07 g; 11.6 mmol) dissolved in CH_3CN (20 mL) is cooled to 5 °C. This solution is added dropwise to sulfanilamide (2.00 g; 11.6 mmol) dissolved in 30 mL CH_3CN. The reactive mixture is allowed to reach room temperature (25 °C) and stirred for 2 h. The solvents are evaporated under reduced pressure, and the crude residue is purified by column chromatography using silica as stationary phase and cyclohexane/ethyl acetate (1:1) as mobile phase. Yield: 33%. Mp: 212 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 7.49

(s, 2H, NH_2), 7.61 (d, 2H, 3H- and 5H-Ph, $J = 8.80$ Hz), 7.86 (d, 2H, 2H- and 6H-Ph $J = 8.80$ Hz). ^{13}C NMR (DMSO-d_6) δ ppm: 126.42 (2C, C2C6-Ph), 126.71 (2C, C3C5-Ph), 133.30 (C1-Ph), 139.55 (C4-Ph), 179.85 (CS). IR (KBr): 3357 (ν_{as} NH), 3253 (ν_s NH), 2197-2117 (ν N=C=S), 1583 (ν C–C), 1331-1283 (ν_{as} SO_2), 1151 (ν_s SO_2) cm^{-1}. Anal. Calcd for $C_7H_6N_2O_2S_2$: C, 39.24; H, 2.82; N, 13.07; S, 29.93. Found: C, 39.17; H, 2.79; N, 12.95; S, 30.19.

5.1.1.4. General procedure for the synthesis of (±)3,5-diphenyl-2-thioxoimidazolidin-4-ones (5–23). D,L-(±)-α-Phenylglycine (1.5 g; 9.92 mmol) is suspended in 60 mL of a mixture of H_2O/pyridine (1:1), warmed to 40 °C and pH adjusted to 9 with NaOH 1 N. The required isothiocyanate (14.9 mmol) is added portionwise over 1 h under stirring. During this period the pH value is maintained at 9. Thirty minutes later, the solvents are evaporated under reduced pressure, and the crude residue is dissolved in water (100 mL). The solution is extracted three times with toluene (3×50 mL) and then acidified with HCl (1 N). The formed precipitate is extracted by ethyl acetate (3×50 mL). The organic layers are combined, dried and the solvent evaporated. The resulting residue is then crystallized from ethanol to afford the (±)3,5-diaryl-2-thioxoimidazolidin-4-ones (**5**–**23**).

5.1.1.5. (±)3,5-Diphenyl-2-thioxoimidazolidin-4-one (5). Yield: 37%. Mp: 232–233 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.60 (d, 1H, CH-CO, $J = 1.20$ Hz); 7.31–7.53 (m, 10H, 2 Ph); 11.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.80 (C5-heterocycle), 127.29 (2C, C3C5-Ph), 128.87, (2C, C2C6-Ph-N), 128.94 (C4-Ph), 129.02 (2C, C3C5-Ph-N), 129.17 (2C, C2C6-Ph), 129.37 (C4-Ph) 133.38 (C1-Ph-N), 134.58 (C1-Ph), 172.92 (CO), 182.87 (CS). IR (KBr): 3147 (ν NH), 1758 (ν C=O), 1596 (ν C–C), 1520 (thioureide), 1404 (ν_{as} NCS), 1273–1246 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44; S, 11.95. Found: C, 67.04; H, 4.68; N, 10.53; S, 11.67.

5.1.1.6. (±)3-(4-Methylphenyl)-5-phenyl-2-thioxoimidazolidin-4-one (6). Yield: 62%. Mp: 237–238 °C. ^1H NMR (DMSO-d_6) δ ppm: δ 2.35 (s, 3H, CH_3), 5.57 (s, 1H, CH-CO), 7.18 (d, 2H, 3H-Ph-N and 5H-Ph, $J = 12.4$ Hz), 7.29 (d, 2H, 2H-Ph-N and 6H-Ph-N, $J = 13.2$ Hz), 7.38–7.47 (m, 5H, Ph), 10.9 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 20.78 (CH_3), 62.77 (C5-heterocycle), 127.27 (2C, C3C5-Ph), 128.77 (2C, C2C6-Ph-N), 128.99 (C4-Ph), 129.19 (2C, C2C6-Ph), 129.50 (2C, C3C5-Ph-N), 130.88 (C4-Ph-N), 134.64 (C1-Ph), 138.49 (C1-Ph-N), 173.01 (CO), 183.16 (CS). IR (KBr): 3176 (ν NH), 1761 (ν C=O), 1590 (ν C–C), 1523 (thioureide), 1407 (ν_{as} NCS), 1251 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92; S, 11.36. Found: C, 67.70; H, 5.40; N, 10.09; S, 11.61.

5.1.1.7. (±)3-(4-Ethylphenyl)-5-phenyl-2-thioxoimidazolidin-4-one (7). Yield: 47%. Mp: 228–229 °C. ^1H NMR (DMSO-d_6) δ ppm: 1.21 (t, 3H, CH_3, $J = 7.50$ Hz), 2.66 (q, 2H, CH_2, $J = 7.50$ Hz), 5.58 (s, 1H, CH-CO), 7.09–7.43 (m, 9H, 2 Ph), 10.9 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 15.30 (CH_3), 27.64 (CH_2), 62.77 (C5-heterocycle), 127.26 (2C, C3C5-Ph), 128.31 (2C, C2C6-Ph-N), 128.75 (2C, C3C5-Ph-N), 128.98 (C4-Ph), 129.19 (2C, C2C6-Ph), 131.08 (C4-Ph-N), 134.63 (C1-Ph), 144.58 (C1-Ph-N), 173.02 (CO), 183.15 (CS). IR (KBr): 3174 (ν NH), 1760 (ν C=O), 1603 (ν C–C), 1523 (thioureide), 1407 (ν_{as} NCS), 1275 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_2OS$: C, 68.89; H, 5.44; N, 9.45. Found: C, 68.67; H, 5.25; N, 9.72.

5.1.1.8. (±)3-(4-Fluorophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (8). Yield: 51%. Mp: 227–228 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.58 (s, 1H, CH-CO), 7.32–7.64 (m, 9H, 2 Ph), 11.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.85 (C5-heterocycle), 115.78 (2C, C3C5-Ph-N), 127.39 (2C, C3C5-Ph), 129.02 (C4-Ph), 129.19 (2C, C2C6-Ph), 131.25 (2C, C2C6-Ph-N), 131.34 (C1-Ph-N), 134.50 (C1-Ph), 160.78 (C4-Ph-N), 172.93 (CO), 182.86 (CS). IR (KBr): 3167 (ν NH), 1757 (ν C=O), 1601 (ν C–C), 1523 (thioureide), 1407 (ν_{as} NCS), 1275–1248 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}FN_2OS$: C, 62.92; H, 3.87; N, 9.78; S, 11.20. Found: C, 63.19; H, 3.99; N, 9.73; S, 11.46.

5.1.1.9. (±)3-(4-Chlorophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (9). Yield: 46%. Mp: 219–220 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.58 (s, 1H, CH-CO), 7.33–7.63 (m, 9H, 2 Ph), 11.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.97 (C5-heterocycle), 127.42 (2C, C3C5-Ph), 129.08 (C4-Ph), 129.22 (2C, C2C6-Ph), 131.00 (2C, C3C5-Ph-N), 131.25 (2C, C2C6-Ph-N), 132.40 (C1-Ph-N), 133.59 (C4-Ph-N), 134.46 (C1-Ph), 172.79 (CO), 182.61 (CS). IR (KBr): 3168 (ν NH), 1763 (ν C=O), 1595 (ν C–C), 1520 (thioureide), 1407 (ν_{as} NCS), 1276–1249 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}ClN_2OS$: C, 59.50; H, 3.66; N, 9.25; S, 10.59. Found: C, 59.24; H, 3.68; N, 9.31; S, 10.59.

5.1.1.10. (±)3-(4-Bromophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (10). Yield: 26%. Mp: 224 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.60 (s, 1H, CH-CO), 7.34 (d, 2H, 2H-Ph and 6H-Ph, $J = 9{,}30$ Hz), 7.41–7.50 (m, 5H, Ph), 7.72 (d, 2H, 3H-Ph and 5H-Ph, $J = 8{,}90$ Hz), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.94 (C5-heterocycle), 122.12 (C4-Ph-N), 127.37 (2C, C3C5-Ph), 129.04, (C4-Ph), 129.18 (2C, C2C6-Ph), 131.24 (2C, C2C6-Ph-N), 132.01 (2C, C3C5-Ph-N), 132.79 (C1-Ph-N), 134.42 (C1-Ph), 172.71 (CO), 182.50 (CS). IR (KBr): 3158 (ν NH), 1755 (ν C=O), 1590 (ν C–C), 1522 (thioureide), 1404 (ν_{as} NCS), 1276–1246 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}BrN_2OS$: C, 51.88; H, 3.19; N, 8.07; S, 9.23. Found: C, 52.49; H, 3.15; N, 7.80; S, 8.61.

5.1.1.11. (±)3-(4-Iodophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (11). Yield: 24%. Mp: 242–243 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.58 (s, 1H, CH-CO), 7.16 (dt, 2H, 2H-Ph and 6H-Ph $J = 6.60$ and 1.60 Hz), 7.39–7.46 (m, 5H, phenyl), 7.86 (dt, 2H, 3H-Ph and 5H-Ph, $J = 6.80$ and 2.00 Hz), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.92 (C5-heterocycle), 95.32 (C4-Ph-N), 127.33 (2C, C3C5-Ph), 129.04, (C4-Ph), 129.18 (2C, C2C5-Ph), 131.26 (2C, C2C6-Ph-N),

133.26 (C1-Ph-N), 134.42 (C1-Ph), 137.90 (2C, C3C5-Ph-N), 172.71 (CO), 182.51 (CS). IR (KBr): 3159 (ν NH), 1756 (ν C=O), 1585 (ν C–C), 1522 (thioureide), 1405 (ν_{as} NCS), 1276–1245 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}IN_2OS$: C, 45.70; H, 2.81; N, 7.11; S, 8.13. Found: C, 46.22; H, 2.68; N, 7.29; S, 7.52.

5.1.1.12. (±)5-Phenyl-2-thioxo-3-[4-(trifluoromethyl)phenyl]imidazolidin-4-one (12). Yield: 42%. Mp: 223–224 °C. ^1H NMR (DMSO-d_6) δ ppm: δ 5.62 (s, 1H, CH-CO), 7.45 (s, 5H, Ph), 7.63 (d, 2H, 2H-Ph and 6H-Ph, J = 8.10 Hz), 7.90 (d, 2H, 3H-Ph and 5H-Ph, J = 8.60 Hz), 11.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.91 (C5-heterocycle), 125.59 (CF_3), 127.31 (2C, C3C5-Ph), 128.83 (2C, C2C6-Ph-N), 128.97 (C4-Ph), 129.19 (2C, C2C6-Ph), 129.71 (C4-Ph-N), 129.86 (2C, C3C5-Ph-N), 134.13 (C1-Ph), 136.94 (C1-Ph-N), 172.36 (CO), 181.95 (CS). IR (KBr): 3158 (ν NH), 1759 (ν C=O), 1615 (ν C–C), 1526 (thioureide), 1406 (ν_{as} NCS), 1274–1246 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{11}F_3N_2$ OS: C, 57.14; H, 3.30; N, 8.33; S, 9.53. Found: C, 57.47; H, 3.49; N, 8.51; S, 9.57.

5.1.1.13. (±)3-(4-Nitrophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (13). Yield: 25%. Mp: 181–182 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.63 (d, 1H, CH-CO, J = 1.10 Hz), 7.45 (s, 5H, Ph), 7.71 (d, 2H, 2H-Ph and 6H-Ph, J = 9.01 Hz), 8.36 (d, 2H, 3H-Ph and 5H-Ph, J = 9.23 Hz), 11.2 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 63.15 (C5-heterocycle), 124.18 (2C, C2C6-Ph-N), 127.48 (2C, C3C5-Ph), 129.13 (C4-Ph), 129.22 (2C, C2C6-Ph), 130.46 (2C, C3C5-Ph-N), 134.23 (C1-Ph), 139.11 (C4-Ph-N), 147.35 (C1-Ph), 172.49 (CO), 181.85 (CS). IR (KBr): 3178 (ν NH), 1766 (ν C=O), 1614–1595 (ν C–C), 1535 (thioureide), 1403 (ν_{as} NCS), 1246 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}N_3O_3S$: C, 57.50; H, 3.54; N, 13.41; S, 10.23. Found: C, 57.72; H, 3.94; N, 13.44; S, 9.85.

5.1.1.14. (±)3-[4-(Methylsulfonyl)phenyl]-5-phenyl-2-thioxoimidazolidin-4-one (14). Yield: 15%. Mp: 280–281 °C. ^1H NMR (DMSO-d_6) δ ppm: δ 3.30 (s, 3H, CH_3), 5.62 (s, 1H, CH-CO), 7.42–7.47 (m, 5H, Ph), 7.68 (d, 2H, 2H-Ph and 6H-Ph, J = 8.80 Hz), 8.06 (d, 2H, 3H-Ph and 5H-Ph, J = 8.40 Hz), 11.2 (s, 1H, NH). δ ppm: 43.77 (CH_3), 63.10 (C5-heterocycle), 127.46 (2C, C3C5-Ph), 127.90, (2C, C2C6-Ph-N), 129.13 (C4-Ph), 129.22 (2C, C2C6-Ph), 130.15 (2C, C3C5-Ph-N), 134.31 (C1-Ph), 137.88 (C4-Ph-N), 141.02 (C1-Ph-N), 172.64 (CO), 182.09 (CS). IR (KBr): 3305 (ν NH_{sulfo}), 3022–3005 (ν NH), 1755 (ν C=O), 1596 (ν C–C), 1509 (thioureide), 1403 (ν_{as} NCS), 1252 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{14}N_2O_3$ S_2: C, 55.47; H, 4.07; N, 8.09; S, 18.51. Found: C, 54.68; H, 3.87; N, 8.10; S, 18.31.

5.1.1.15. (±)3-[4-(Aminosulfonyl)phenyl]-5-phenyl-2-thioxoimidazolidin-4-one (15). Yield: 25%. Mp: >350 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.63 (s, 1H, CH-CO), 7.42–7.50 (m, 7H, Ph and NH_2), 7.58 (d, 2H, 2H-Ph and 6H-Ph, J = 8.00 Hz), 7.94 (d, 2H, 3H-Ph and 5H-Ph, J = 8.40 Hz), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 63.02 (C5-heterocycle), 126.43 (2C, C2C6-Ph-N), 127.38, (2C, C3C5-Ph), 129.08 (C4-Ph), 129.21 (2C, C2C6-Ph), 129.76 (2C, C3C5-Ph-N), 134.35 (C1-Ph), 136.28 (C4-Ph-N), 144.32 (C1-Ph-N), 172.70 (CO), 182.32 (CS). IR (KBr): 3360 (ν NH_{sulfo}), 3063–3043 (ν NH), 1759 (ν C=O), 1596 (ν C–C), 1512 (thioureide), 1406 (ν_{as} NCS), 1269 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{13}N_3O_3S_2$: C, 51.86; H, 3.77; N, 12.10; S, 18.46. Found: C, 51.61; H, 3.76; N, 11.97; S, 18.18.

5.1.1.16. (±)3-(3-Methylphenyl)-5-phenyl-2-thioxoimidazolidin-4-one (16). Yield: 47%. Mp: 218–219 °C. ^1H NMR (DMSO-d_6) δ ppm: 2.34 (s, 3H, CH_3), 5.59 (d, 1H, CH-CO, J = 1.32 Hz), 7.12–7.46 (m, 9H, 2 Ph), 10.9 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 20.76 (CH_3), 62.81 (C5-heterocycle), 126.13 (C6-Ph-N), 127.30 (2C, C3C5-Ph), 128.81 (C2-Ph-N), 129.00 (C4-Ph), 129.22 (2C, C2C6-Ph), 129.37 (C4-Ph-N), 129.61 (C5-Ph-N), 133.44 (C3-Ph-N), 134.63 (C1-Ph), 138.54 (C1-Ph-N), 172.77 (CO), 183.05 (CS). IR (KBr): 3174 (ν NH), 1757 (ν C=O), 1609–1591 (ν C–C), 1518 (thioureide), 1402 (ν_{as} NCS), 1274–1254 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92; S, 11.36. Found: C, 67.82; H, 5.33; N, 10.14; S, 11.40.

5.1.1.17. (±)3-(3-Methoxyphenyl)-5-phenyl-2-thioxoimidazolidin-4-one (17). Yield: 26%. Mp: 197–199 °C. ^1H NMR (DMSO-d_6) δ ppm: 3.77 (s, 3H, CH_3), 5.57 (s, 1H, CH-CO), 6.85–7.09 (m, 3H, Ph), 7.32–7.50 (m, 6H, Ph), 10.9 (s, 1H, NH). δ ppm: 55.44 (CH_3), 62.83 (C5-heterocycle), 114.48 (C2-Ph-N), 114.93 (C4-Ph-N), 121.18 (C6-Ph-N), 127.30 (C5-Ph-N), 127.36 (2C, C3C5-Ph), 128.97 (C4-Ph), 129.18 (2C, C2C6-Ph), 129.72 (C1-Ph-N), 134.56 (C1-Ph), 159.62 (C3-Ph-N), 172.82 (CO), 182.90 (CS). IR (KBr): 3159 (ν NH), 1754 (ν C=O), 1607 (ν C–C), 1518 (thioureide), 1404 (ν_{as} NCS), 1272 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39; S, 10.75. Found: C, 64.42; H, 5.01; N, 9.58; S, 10.56.

5.1.1.18. (±)3-(3-Fluorophenyl-5-phenyl)-2-thioxoimidazolidin-4-one (18). Yield: 35%. Mp: 223–224 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.58 (s, 1H, CH-CO), 7.03–7.59 (m, 9H, 2 Ph), 11.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.86 (C5-heterocycle), 111.28 (C2-Ph-N), 113.87 (C4-Ph-N), 115.42 (C6-Ph-N), 127.49 (2C, C3C5-Ph), 129.03 (C4-Ph), 129.17 (2C, C2C6-Ph), 133.98 (C5-Ph-N), 134.47 (C1-Ph), 137.13 (C1-Ph-N), 161.21 (C3-Ph-N), 172.91 (CO), 182.51 (CS). IR (KBr): 3180 (ν NH), 1760 (ν C=O), 1600 (ν C–C), 1518 (thioureide), 1405 (ν_{as} NCS), 1276–1254 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}FN_2OS$: C, 62.92; H, 3.87; N, 9.78; S, 11.20. Found: C, 63.28; H, 4.21; N, 9.99; S, 11.09.

5.1.1.19. (±)3-(3-Chlorophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (19). Yield: 23%. Mp: 201–202 °C. ^1H NMR (DMSO-d_6) δ ppm: 5.58 (s, 1H, CH-CO), 7.32–7.57 (m, 9H, 2 Ph), 11.1 (s, 1H, NH). δ ppm: 62.99 (C5-heterocycle), 123.82 (C6-Ph-N), 125.99 (C2-Ph-N), 127.51 (2C, C3C5-Ph), 128.05 (C4-Ph-N), 129.03 (C4-Ph), 129.15 (2C, C2C6-Ph), 130.57 (C5-Ph-N), 132.98 (C3-Ph-N), 134.38 (C1-Ph), 134.83 (C1-Ph-N), 172.69 (CO), 182.42 (CS). IR (KBr): 3161 (ν NH), 1766 (ν C=O), 1592 (ν C–C), 1519 (thioureide), 1399 (ν_{as} NCS), 1276–1244 (ν_s NCS) cm^{-1}. Anal. Calcd for

$C_{15}H_{11}ClN_2OS$: C, 59.50; H, 3.66; N, 9.25; S, 10.59. Found: C, 59.58; H, 3.86; N, 9.42; S, 10.52.

5.1.1.20. (±)3-(3-Bromophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (20). Yield: 32%. Mp: 197 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 5.57 (s, 1H, CH-CO), 7.44–7.65 (m, 9H, 2 Ph), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.93 (C5-heterocycle), 124.23 (C6-Ph-N), 127.50 (2C, C3C5-Ph), 128.23 (C3-Ph-N), 128.75 (C2-Ph-N), 129.05 (C4Ph), 129.17 (2C, C2C6-Ph), 130.89 (C5-Ph-N), 131.23 (C4-Ph-N), 134.51 (C1-Ph), 138.12 (C1-Ph-N), 172.83 (CO), 182.47 (CS). IR (KBr): 3169 (ν NH), 1762 (ν C=O), 1576 (ν C–C), 1518 (thioureide), 1402 (ν_{as} NCS), 1273 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}BrN_2OS$: C, 51.89; H, 3.19; N, 8.07; S, 9.23. Found: C, 51.71; H, 3.27; N, 8.30; S, 9.34.

5.1.1.21. (±)3-(3-Iodophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (21). Yield: 28%. Mp: 199 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 5.56 (s, 1H, CH-CO), 7.30 (t, 1H, 3H-Ph, J = 7.60 Hz), 7.37–7.46 (m, 6H, 2H-Ph and Ph), 7.77 (t, 1H, 6H-Ph, J = 1.80 Hz), 7.82 (dt, 1H, 4H-Ph, J = 8.00 Hz and J = 1.60 Hz), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 62.98 (C5-heterocycle), 93.98 (C3-Ph-N), 127.51 (2C, C3C5-Ph), 128.77 (C2-Ph-N), 129.03 (C4-Ph), 129.16 (2C, C2C6-Ph), 130.86 (C6-Ph-N), 134.37 (C5-Ph-N), 134.72 (C1-Ph), 137.45 (C4-Ph-N), 137.65 (C1-Ph-N), 172.74 (CO), 182.49 (CS). IR (KBr): 3172 (ν NH), 1758 (ν C=O), 1585–1574 (ν C–C), 1517 (thioureide), 1423 (ν_{as} NCS), 1272–1245 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{15}H_{11}IN_2OS$: C, 45.70; H, 2.81; N, 7.11; S, 8.13. Found: C, 46.11; H, 2.79; N, 7.36; S, 7.43.

5.1.1.22. (±)5-Phenyl-2-thioxo-3-[3-(trifluoromethyl)phenyl]imidazolidin-4-one (22). Yield: 14%. Mp: 191–192 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 5.60 (s, 1H, CH-CO), 7.45 (s, 5H, Ph), 7.72–7.84 (m, 4H, Ph), 11.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 63.13 (C5-heterocycle), 125.44 (C2-Ph-N), 125.78 (CF_3), 126.10 (C4-Ph-N), 126.18 (C6-Ph-N), 127.62 (2C, C3C5-Ph), 129.10 (C4-Ph), 129.19 (2C, C2C6-Ph), 130.28 (C5-Ph-N), 133.46 (C3-Ph-N), 134.29 (C1-Ph), 134.44 (C1-Ph-N), 172.78 (CO), 182.41 (CS). IR (KBr): 3186 (ν NH), 1770 (ν C=O), 1598 (ν C–C), 1517 (thioureide), 1406 (ν_{as} NCS), 1275–1249 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{11}F_3N_2OS$: C, 57.14; H, 3.30; N, 8.33; S, 9.53. Found: C, 56.81; H, 3.73; N, 8.45; S, 9.96.

5.1.1.23. (±)3-[3-(Methylsulfonyl)phenyl]-5-phenyl-2-thioxoimidazolidin-4-one (23). Yield: 19%. Mp: 248–249 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.28 (s, 3H, CH_3), 5.61 (s, 1H, CH-CO), 7.41–7.50 (m, 5H, Ph), 7.75–7.83 (m, 2H, 2H-Ph and 3H-Ph), 7.99–8.03 (m, 2H, 4H-Ph and 6H-Ph), 11.2 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 43.52 (CH_3), 63.02 (C5-heterocycle), 124.64 (C2-Ph-N), 125.73 (C4-Ph-N), 126.02 (C6-Ph-N), 127.49 (2C, C3C5-Ph), 129.08 (C4-Ph), 129.20 (2C, C2C6-Ph), 129.86 (C5-Ph-N), 134.33 (C1-Ph), 134.56 (C3-Ph-N), 135.21 (C1-Ph-N), 172.68 (CO), 182.35 (CS). IR (KBr): 3271 (ν NH), 3053 (ν NH), 3018 (ν NH), 1763 (ν C=O), 1599 (ν C–C), 1516 (thioureide), 1403 (ν_{as} NCS), 1264 (ν_s NCS) cm^{-1}. Anal. Calcd for $C_{16}H_{14}N_2O_3S_2$: C, 55.47; H, 4.07; N, 8.09; S, 18.51. Found: C, 55.22; H, 4.12; N, 8.20; S, 18.56.

5.2. Inhibition of isolated COXs

A COX inhibitor screening assay (Cayman Chemical Company, Ann Arbor, MI, USA) has been used to determine the activity of isolated ovine COX-1 and human recombinant COX-2. Briefly, COX (1000 UI mL^{-1}) is incubated with the studied drug (50 μM) at 37 °C for 5 min in a Tris–HCl buffer (0.1 M, pH 8) containing EDTA (5 mM), phenol (2 mM) and haem (500 μM). Drugs are dissolved in DMSO. Then, the COX reaction is initiated by addition of arachidonic acid (10 mM). The reaction is stopped 2 min later by HCl 1 N (50 μL), followed by addition of saturated $SnCl_2$ (0.1 mL) to reduce the COX-produced PGH_2 into $PGF_{2\alpha}$ which was further quantified by EIA using PGE_2 as standard. For each drug, the inhibitory potency is expressed as follows: COX inhibition (%) = $100 \times ([PGF_{2\alpha}] - [PGF_{2\alpha}]_d)/[PGF_{2\alpha}]$ where $[PGF_{2\alpha}]$ and $[PGF_{2\alpha}]_d$ are the amounts produced in absence and in presence of the studied drug, respectively. Blank was previously subtracted to each value. Results are means of three independent experiments.

5.3. Molecular modelling

Homology modelling of the human COX-2 from murine crystallized COX-2 (PDB entry: 6COX) was performed using the package *Homology* in *InsightII*.[43] The algorithm *Research*[38] performs exploration of one region of the protein (rigid) by one ligand (flexible). The hypothesis generation is based on a Monte Carlo algorithm, randomly generating conformations. Cutoff: 15 Å; Ecut: −10 kcal/mol; *n* trials: 10,000. *Gold* is a genetic algorithm for docking flexible ligands into protein binding sites.[37] Conformation of some amino acids (Ser, Thr and Lys) is optimized during the run. Popsiz = 100; maxops = 100,000; niche_size = 2. *Autodock* uses a hybrid method called Lamarckian Genetic Algorithm (genetic algorithm coupled with a local search) to predict the interaction of ligands with macromolecular targets.[36] Runs, 200; population size, 50; number of generations, 27,000. *Discover3* uses the molecular mechanics to optimize the conformation of the ligand-protein complex and evaluate the interaction energy (ΔEvdw and ΔEcb). The backbone is moved following force constants and side chains move freely.[39] Forcefield, CVFF; dielectric constant, 1∗*r*; criteria convergence, 10 kcal/mol for the Steepest Descent algorithm, 0.01 for the Conjugate Gradient one.

5.4. Whole blood assay

The human whole blood was assayed for COX activity according to a similar procedure previously described.[44] For COX-1 activity measurement, human fresh blood (250 μL) collected by venipuncture in heparinized tubes is incubated under constant agitation for 15 min at 37 °C with the investigated drug dissolved in DMSO (2 μL). COX-1 is then activated by calcimycin addition (20 μM, 10 μL). Ten minutes later, blood is centrifuged (10,000*g*, 10 min, 4 °C) and supernatant collected for

TXB_2 dosage using an enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA). For COX-2 activity measurement, the drug (2 μL) is also incubated for 15 min (37 °C). At the end of this incubation period, lipopolysaccharide (100 μg mL^{-1}) is added and incubated for 24 h. Then blood is centrifuged (10,000*g*, 10 min, 4 °C) and supernatant collected for PGE_2 dosage by using an enzyme immunoassay kit according to the manufacturer's instructions. (Cayman Chemical Company, Ann Arbor, MI, USA). The COX inhibitory potency is expressed as follows: inhibition (%) = $100 \times (1-(P-P_0)/(P_{max}- P_0))$ where P and P_{max} are the prostanoid production (expressed as TXB_2 for COX-1 activity and PGE_2 for COX-2 activity) in the presence and in the absence of drug, respectively. P_0 is the basal prostanoid production in the absence of drug and inducer. Values are means of three independent experiments performed in triplicate.

5.5. Stability

The stability of compounds **5**, **12**, **20** and **23** in water was monitored by measuring the concentration at several times using LC/MS. The half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = (\ln 2)/k$ where k is the first-order kinetic constant. k value was obtained by nonlinear regression fits of the experimental data to the equations $A=A_0 \cdot e^{-kt}$ using GraphPad Prism 3.0 software. The concentration during the course of degradation was measured by injection of 10 μL of compounds **5**, **12**, **20** and **23** (initial concentration 50 μM) into an Agilent SB-C18 column, pore size 80 Å, 100 × 3 mm i.d. at 25 °C. Solvent A consisted of water with 0.1% formic acid and solvent B of acetonitrile with 0.1% formic acid. The gradient profile for solvent B was 20–80% in 15 min. The flow rate was 500 μL min^{-1}. After elution from the column, the sample was ionized by ESI source in positive mode of the mass spectrometer. Experiments were performed on an Agilent 1100 LC/MSD Trap (Agilent technologies) operating in MRM mode. Ionization parameters were: nebulizer pressure 60 psi, auxiliary nitrogen flow 10 mL min^{-1}, auxiliary nitrogen temperature 320 °C, and capillary voltage 3.5 kV.

Acknowledgment

This work was partially financed by a grant of the Communauté française de Belgique (ARC 99/04-249).

References and notes

1. Smith, W. L. The eicosanoids and their biochemical mechanisms of action. *Biochem. J.* **1989**, *259*, 315.
2. Lee, S. H.; Soyoola, E.; Chanmugam, P.; Hart, S.; Sun, W.; Zhong, H.; Liou, S.; Simmons, D.; Hwang, D. Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. *J. Biol. Chem.* **1992**, *267*, 25934.
3. Maier, J. A.; Hla, T.; Maciag, T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. *J. Biol. Chem.* **1990**, *265*, 10805.
4. Sirois, J.; Richards, J. S. Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles. *J. Biol. Chem.* **1992**, *267*, 6382.
5. Kawaguchi, H.; Pilbeam, C. C.; Gronowicz, G.; Abreu, C.; Fletcher, B. S.; Herschman, H. R.; Raisz, L. G.; Hurley, M. M. Transcriptional induction of prostaglandin G/H synthase-2 by basic fibroblast growth factor. *J. Clin. Invest.* **1995**, *96*, 923.
6. Xie, W.; Herschman, H. R. Transcriptional regulation of prostaglandin synthase 2 gene expression by platelet-derived growth factor and serum. *J. Biol. Chem.* **1996**, *271*, 31742.
7. Xie, W. L.; Chipman, J. G.; Robertson, D. L.; Erikson, R. L.; Simmons, D. L. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2692.
8. Dannenberg, A. J.; Altorki, N. K.; Boyle, J. O.; Dang, C.; Howe, L. R.; Weksler, B. B.; Subbaramaiah, K. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. *Lancet Oncol.* **2001**, *2*, 544.
9. Shiff, S. J.; Shivaprasad, P.; Santini, D. L. Cyclooxygenase inhibitors: drugs for cancer prevention. *Curr. Opin. Pharmacol.* **2003**, *3*, 352.
10. Leahy, K. M.; Koki, A. T.; Masferrer, J. L. Role of cyclooxygenases in angiogenesis. *Curr. Med. Chem.* **2000**, *7*, 1163.
11. Masferrer, J. L.; Leahy, K. M.; Koki, A. T.; Zweifel, B. S.; Settle, S. L.; Woerner, B. M.; Edwards, D. A.; Flickinger, A. G.; Moore, R. J.; Seibert, K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* **2000**, *60*, 1306.
12. Hoozemans, J. J.; Veerhuis, R.; Rozemuller, A. J.; Eikelenboom, P. Non-steroidal anti-inflammatory drugs and cyclooxygenase in Alzheimer's disease. *Curr. Drug Targets* **2003**, *4*, 461.
13. Teismann, P.; Tieu, K.; Choi, D. K.; Wu, D. C.; Naini, A.; Hunot, S.; Vila, M.; Jackson-Lewis, V.; Przedborski, S. Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5473.
14. Dannhardt, G.; Kiefer, W. Cyclooxygenase inhibitors—current status and future prospects. *Eur. J. Med. Chem.* **2001**, *36*, 109.
15. Uddin, M. J.; Praveen Rao, P. N.; McDonald, R.; Knaus, E. E. A new class of acyclic 2-alkyl-1,1,2-triaryl (*Z*)-olefins as selective cyclooxygenase-2 inhibitors. *J. Med. Chem.* **2004**, *7*, 6108.
16. Bertenshaw, S. R.; Talley, J. J.; Rogier, D. J.; Graneto, M. J.; Rogers, R. S.; Kramer, S. W.; Penning, T.; Koboldt, C. M.; Veenhuizen, A. W.; Zhang, Y.; Perkins, W. E. 3,4-Diarylthiophenes are selective COX-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2919.
17. Li, C. S.; Black, W. C.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W. A.; Claveau, D.; Gauthier, J. Y.; Gordon, R.; Greig, G.; Grimm, E.; Guay, J.; Lau, C. K.; Riendeau, D.; Thérien, M.; Visco, D. M.; Wong, E.; Xu, L.; Prasit, P. A new structural variation on the methanesulfonylphenyl class of selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3181.
18. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. Synthesis and biological evaluation of the 1,5-diary-

lpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J. Med. Chem.* **1997**, *40*, 1347.
19. Hashimoto, H.; Imamura, K.; Haruta, J.; Wakitani, K. 4-(4-Cycloalkyl/aryl-oxazol-5-yl)benzenesulfonamides as selective cyclooxygenase-2 inhibitors: enhancement of the selectivity by introduction of a fluorine atom and identification of a potent, highly selective, and orally active COX-2 inhibitor JTE-522. *J. Med. Chem.* **2002**, *45*, 1511.
20. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *J. Med. Chem.* **2000**, *43*, 775.
21. Talley, J. J.; Bertenshaw, S. R.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Kellogg, M. S.; Koboldt, C. M.; Yuan, J.; Zhang, Y. Y.; Seibert, K. *N*-[[(5-Methyl-3-phenylisoxazol-4-yl)-phenyl]sulfonyl]propanamide, sodium salt, parecoxib sodium: a potent and selective inhibitor of COX-2 for parenteral administration. *J. Med. Chem.* **2000**, *43*, 1661.
22. Carter, J. S.; Kramer, S.; Talley, J. J.; Penning, T.; Collins, P.; Graneto, M. J.; Seibert, K.; Koboldt, C. M.; Masferrer, J.; Zweifel, B. Synthesis and activity of sulfonamide-substituted 4,5-diaryl thiazoles as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1171.
23. Almansa, C.; Alfon, J.; de Arriba, A. F.; Cavalcanti, F. L.; Escamilla, I.; Gomez, L. A.; Miralles, A.; Soliva, R.; Bartroli, J.; Carceller, E.; Merlos, M.; Garcia-Rafanell, J. Synthesis and structure–activity relationship of a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. *J. Med. Chem.* **2003**, *46*, 3463.
24. Khanna, I. K.; Weier, R. M.; Yu, Y.; Xu, X. D.; Koszyk, F. J.; Collins, P. W.; Koboldt, C. M.; Veenhuizen, A. W.; Perkins, W. E.; Casler, J. J.; Masferrer, J. L.; Zhang, Y. Y.; Gregory, S. A.; Seibert, K.; Isakson, P. C. 1,2-Diarylimidazoles as potent, cyclooxygenase-2 selective, and orally active antiinflammatory agents. *J. Med. Chem.* **1997**, *40*, 1634.
25. Dannhardt, G.; Kiefer, W.; Kramer, G.; Maehrlein, S.; Nowe, U.; Fiebich, B. The pyrrole moiety as a template for COX-1/COX-2 inhibitors. *Eur. J. Med. Chem.* **2000**, *35*, 499.
26. Puig, C.; Crespo, M. I.; Godessart, N.; Feixas, J.; Ibarzo, J.; Jimenez, J. M.; Soca, L.; Cardelus, I.; Heredia, A.; Miralpeix, M.; Puig, J.; Beleta, J.; Huerta, J. M.; Lopez, M.; Segarra, V.; Ryder, H.; Palacios, J. M. Synthesis and biological evaluation of 3,4-diaryloxazolones: a new class of orally active cyclooxygenase-2 inhibitors. *J. Med. Chem.* **2000**, *43*, 214.
27. Friesen, R. W.; Brideau, C.; Chan, C. C.; Charleson, S.; Deschenes, D.; Dube, D.; Ethier, D.; Fortin, R.; Gauthier, J. Y.; Girard, Y.; Gordon, R.; Greig, G.; Riendeau, D.; Savoie, C.; Wang, Z.; Wong, E. 2-Pyridinyl-3-(4-methylsulfonyl)phenylpyridines: selective and orally active cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2777.
28. Li, C. S.; Brideau, C.; Chan, C. C.; Savoie, C.; Claveau, D.; Charleson, S.; Gordon, R.; Greig, G.; Gauthier, J. Y.; Lau, C. K.; Riendeau, D.; Therien, M.; Wong, E.; Prasit, P. Pyridazinones as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 597.
29. Joo, Y. H.; Kim, J. K.; Kang, S.-H.; Noh, M. S.; Ha, J.-Y.; Choi, J. K.; Lim, K. M.; Chung, S. 2,3-Diarylpyran-4-ones: a new series of selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2195.
30. Ortiz, E. Market withdrawal of Vioxx: is it time to rethink the use of COX-2 inhibitors? *J. Manag. Care Pharm.* **2004**, *10*, 551.
31. Sibbald, B. Rofecoxib (Vioxx) voluntarily withdrawn from market. *Can. Med. Assoc. J.* **2004**, *171*, 1027.
32. Edman, P. Preparation of phenyl thiohydantoins from some natural amino acids. *Acta Chem. Scand.* **1950**, *4*, 277.
33. Lopez, A. L.; Trigo, G. G. The chemistry of hydantoins. *Adv. Heterocycl. Chem.* **1985**, *38*, 177.
34. Staab, H. A.; Walther, G. Synthese von isothiocyanaten. *Liebigs Ann. Chem.* **1962**, *657*, 104.
35. Michaux, C.; Charlier, C. Structural approach for COX-2 inhibition. *Mini Rev. Med. Chem.* **2004**, *4*, 603.
36. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639.
37. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Gold, 1.2 ed.; Astex Technology, Cambridge, UK.
38. Hart, T. N., Ness, S. R. Dockvision, 1.0.3 ed.; Alberta.
39. Accelrys, Discover3, 2.98 ed.; Accelrys Inc., San Diego.
40. Garcia-Nieto, R.; Perez, C.; Gago, F. Automated docking and molecular dynamics simulations of nimesulide in the cyclooxygenase active site of human prostaglandin-endoperoxide synthase-2 (COX-2). *J. Comput. Aided Mol. Des.* **2000**, *14*, 147.
41. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* **1996**, *384*, 644.
42. Warner, T. D.; Giuliano, F.; Vojnovic, I.; Bukasa, A.; Mitchell, J. A.; Vane, J. R. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7563.
43. Biosym/MSI; InsightII, 97.0 ed.; Homology, Molecular simulations Inc.
44. de Leval, X.; Delarge, J.; Devel, P.; Neven, P.; Michaux, C.; Masereel, B.; Pirotte, B.; David, J. L.; Henrotin, Y.; Dogné, J. M. Evaluation of classical NSAIDs and COX-2 selective inhibitors on purified ovine enzymes and human whole blood. *Prostaglandins Leukot. Essent. Fatty Acids* **2001**, *64*, 211.