Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 795–808
IL FARMACO
www.elsevier.com/locate/farmac

4-Substituted 1,5-diarylpyrazole, analogues of celecoxib: synthesis and preliminary evaluation of biological properties

Giulia Menozzi [a,*], Luisa Merello [a], Paola Fossa [a], Luisa Mosti [a], Antonietta Piana [b], Francesca Mattioli [c]

[a] Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genova, Italy
[b] Dipartimento di Medicina Interna, Centro di Ricerca sulla Trombosi, Università di Genova, Viale Benedetto XV 6, I-16132 Genova, Italy
[c] Dipartimento di Medicina Interna, Sezione di Farmacologia e Tossicologia Clinica, Università di Genova, Viale Benedetto XV 6, I-16132 Genova, Italy

Received 25 November 2002; accepted 29 January 2003

Abstract

A number of 5-aryl-1-[4-(methylsulfonyl)-phenyl]-1*H*-pyrazoles and 4-(5-aryl-1*H*-pyrazol-1-yl)benzenesulfonamides **3**, **4**, **5**, **6**, analogues of the COX-2 selective inhibitor celecoxib (celebrex), were synthesized. In order to verify the effects on the biological properties of certain substituents put on position 4 of the pyrazole nucleus, some of these compounds were screened in vivo for their anti-inflammatory and analgesic activities. Moreover, sodium salts of carboxylic acids **4** were tested in vitro for their platelet anti-aggregating properties. The results of these preliminary biological assays showed that new derivatives are not endowed with improved anti-inflammatory and analgesic properties, in comparison with celecoxib. In addition, docking studies were carried out on the most significative compounds to evaluate their interaction mode at the active site of both COX-1 and COX-2. Some remarks about the SAR of this class of COX-inhibitors are drown out.

Keywords: Pyrazole derivatives; Anti-inflammatory agents; Analgesic agents; COX-inhibitors

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs. The research in this field, in the last years, aimed at discovering new potent agents without unfavourable side effects characteristic of traditional drugs, such as gastrointestinal erosion and renal damage.

It is now known that inhibition of the enzyme cyclooxygenase (COX) is the principal mechanism for both the efficacy and the toxicity of NSAIDs. This enzyme exists as at least two distinct and independently regulated isoenzymes, COX-1 and COX-2. COX-1 is constitutive and synthesizes prostaglandins that mediate normal homeostasis in the gastrointestinal tract, kidneys and platelets, whereas COX-2 is inducible and produces mainly prostaglandins that mediate pain and inflammation. Classical NSAIDs inhibit both the isoforms to different extents, many with a preferential selectivity for COX-1. It is believed that it is the inhibition of COX-1 that causes unfavourable side-effects [1].

A high level of selective COX-2 inhibition represents, therefore, a therapeutic strategy to alleviate pain and inflammation without the untoward gastrointestinal, renal, antiaggregating effects due to the COX-1 inhibition associated to non-selective NSAIDs. Moreover, various epidemiological and laboratory studies have indicated that COX-2 inhibitors may have important therapeutic relevance as anticancer agents (against colorectal and breast cancers, in particular). COX-2 inhibition may also help the prevention of premature labour and even retard the progression of Alzheimer's disease [2–4].

Nevertheless, the therapeutic contribution of COX-2 highly selective blockers has to be more extensively evaluated, particularly as these agents could delay the healing of duodenal ulcers and interfere with several COX-2-induced physiological functions [5,6]. Actually

* Correspondence and reprints.
E-mail address: menozzi@unige.it (G. Menozzi).

0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00136-8

COX-2 appears to be also expressed under basal conditions in many organs, suggesting that this isoenzyme may play a more complex physiological role than was expected [7].

Various classes of selective COX-2 inhibitors have been identified in last few years: in particular, tricyclic molecules bearing a methylsulfonyl or sulfamoyl group (coxibs) have been extensively studied and have proved to be a fertile area for the discovery of ever-new active compounds [8,9]. Celecoxib [10] and rofecoxib [11] (Fig. 1) were the first two agents to be approved in selected markets for the treatment of acute pain and certain inflammatory conditions (osteoarthritis, rheumatoid arthritis).

In particular celecoxib, a 3-substituted 1,5-diarylpyrazole, aroused our interest, given that the synthesis of 1-arylpyrazole compounds with potential anti-inflammatory activity has been a topic of our researches for many years [12].

So, in pursuing our studies and in order to acquire further structure–activity correlations in the field of this novel class of non-ulcerogenic anti-inflammatory agents, we planned the synthesis of a number of 4-substituted 1,5-diarylpyrazoles **3**, **4**, **5**, **6**, as illustrated in Fig. 2.

These derivatives have a 4-sulfamoyl (as celecoxib) or a 4-methylsulfonyl (as rofecoxib) group on the 1-phenyl ring; the second aromatic ring is unsubstituted, *para*-substituted or *meta, para*-disubstituted, bearing in mind what suggested by structure–activity relationship (SAR) studies carried out on various 1,2-diarylheterocyclic inhibitors [10,11,13–15].

Among the numerous 1,5-diarylpyrazoles studied as possible selective COX-2 inhibitors, only few are substituted at the position 4 of the heterocyclic ring, whereas most have a substituent at the position 3 [10]. Thus, we prepared a series of 1,5-diarylpyrazoles bearing a cyano, methoxy- or ethoxycarbonyl, hydroxycarbonyl, hydroxymethyl or formyl function at the position 4, to investigate the effects of such substitutions on the biological properties of this class of compounds.

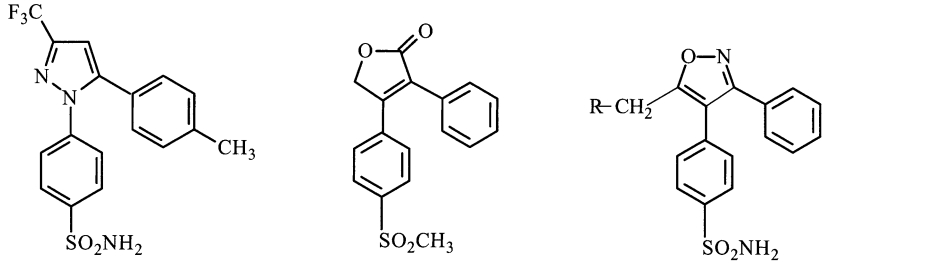
Some remarks guided our choices:

a) The conversion of indomethacin (a well known nonselective NSAID) into ester derivatives generated highly selective COX-2 inhibitors [16].
b) The presence of a hydroxycarbonyl function in the molecule of many traditional NSAIDs, on the contrary, seems to improve their potency against COX-1 [17] and, consequently, it should enhance platelet anti-aggregating properties.
c) A metabolite of the diarylisoxazole derivative valdecoxib, a potent and selective inhibitor of COX-2 recently approved in USA by FDA (Fig. 1), was reported to be more potent than valdecoxib itself in the carrageenan paw edema assay [18]; this metabolite has a hydroxymethyl group on the isoxazole nucleus.

For the synthesis of the new compounds we followed a pathway (Fig. 2) employed in our previous researches which afforded numerous 1-aryl or 1,5-diarylpyrazole derivatives [19–21].

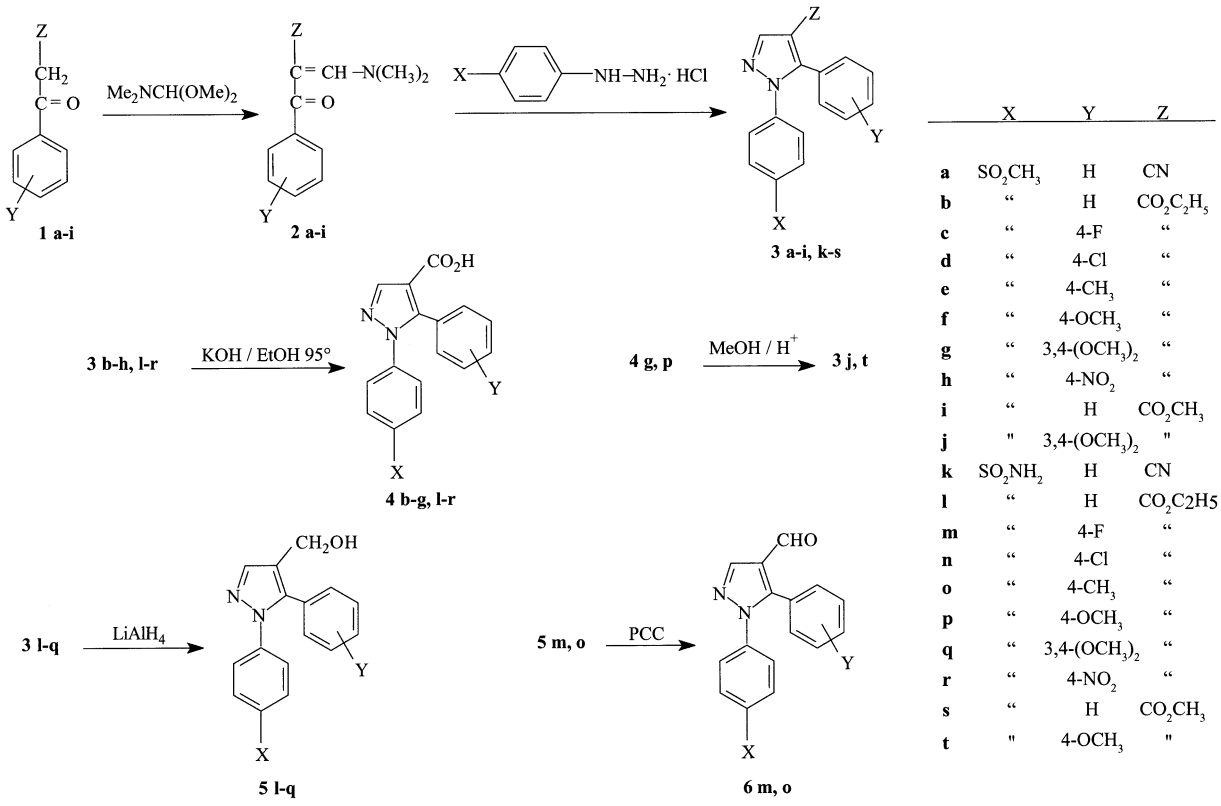
2. Chemistry

Benzoylacetonitrile **1a** and aroylacetates **1b–i** are all known. Some of them are available on the market (**1a**,**b**,**h**), the others can be easily synthesized by the Claisen condensation of the appropriate substituted benzoic ethyl ester with ethyl acetate in the presence of sodium hydride (**1c–g**); methyl ester **1i** was prepared by transesterification of the corresponding ethyl ester **1b**. Treatment of **1a–i** with *N*,*N*-dimethylformamide dimethylacetal provided α-dimethylaminomethylene derivatives (**2a–i**). A survey of the literature revealed that only two of them (**2f**,**i**) are unknown [22–27]. Enaminones (**2a-i**) were condensed with [4-(methylsulfonyl)phenyl]hydrazine or 4-hydrazinobenzenesulfonamide hydrochlorides to afford pyrazoles **3a–i** and **3k–s**, respectively, as sole products in agreement with what already observed [19] and as confirmed by ^{1}H NMR spectra. Esters (**3b–h**,**l–r**) were converted to carboxylic acids (**4b–g**,**l–r**) by alkaline hydrolysis (potassium



celecoxib **rofecoxib** **valdecoxib** (R=H) and its active metabolite (R=OH)

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



	X	Y	Z
a	SO_2CH_3	H	CN
b	"	H	$CO_2C_2H_5$
c	"	4-F	"
d	"	4-Cl	"
e	"	4-CH_3	"
f	"	4-OCH_3	"
g	"	3,4-$(OCH_3)_2$	"
h	"	4-NO_2	"
i	"	H	CO_2CH_3
j	"	3,4-$(OCH_3)_2$	"
k	SO_2NH_2	H	CN
l	"	H	CO_2C2H5
m	"	4-F	"
n	"	4-Cl	"
o	"	4-CH_3	"
p	"	4-OCH_3	"
q	"	3,4-$(OCH_3)_2$	"
r	"	4-NO_2	"
s	"	H	CO_2CH_3
t	"	4-OCH_3	"

Fig. 2. Synthetic routes to compounds **3**–**6**.

hydroxide in ethanol), followed by acidification. In the case of **3h**, it was not possible to isolate the corresponding carboxylic acid from the reaction mixture. Fisher esterification of **4g**,**p** with methanol afforded **3j**,**t**. Lithium aluminum hydride reduction in tetrahydrofuran of ethyl esters (**3l**–**q**) gave corresponding hydroxymethyl derivatives (**5l**–**q**); two of them (**5m**,**o**) were finally oxidized to the corresponding aldehydes (**6m**,**o**) by pyridinium chlorochromate (PCC) in dichloromethane.

3. Pharmacology

Among the compounds prepared by us, we have selected esters **3b**,**e**,**g**,**j**,**o**,**p**,**q**,**t**, carboxylic acids **4g**,**p**, alcohols **5m**–**o**,**q**, aldehyde **6o**, nitriles **3a**,**k** to be evaluated in vivo regarding their anti-inflammatory activity (carrageenan-induced paw edema test in rats) (Table 9).

Nitrile **3k** and esters **3g**,**p**, the most active as anti-inflammatory agents, as well as corresponding methyl esters **3j**,**t** were further assayed in vivo regarding their analgesic effect (writhing test in mice).

Finally, in vitro tests were performed to evaluate the platelet antiaggregating properties of sodium salts of carboxylic acids **4b**–**g**,**l**–**r** (Table 10).

4. Experimental

4.1. Chemistry

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. IR spectra were registered on a Perkin–Elmer 398 spectrophotometer and are expressed in cm^{-1}. ^1H NMR spectra were registered on a Varian Gemini 200 (200 MHz) spectrometer; chemical shifts are reported as δ (ppm) relative to TMS as internal standard; coupling constants (J) are expressed in Hertz (Hz). Microanalyses for C, H, N, S were performed using a Carlo Erba elemental analyzer model EA 1110 and results agree within $\pm 0.3\%$ with calculated values.

4.1.1. General procedure for compounds *1c*–*g*

A solution of appropriate substituted benzoic ethyl ester (10 mmol) in anhydrous benzene (50 ml) was added to sodium hydride (0.80 g of 60% dispersion in paraffin oil, 20 mmol) in anhydrous benzene (50 ml). The mixture was stirred and heated to reflux while ethyl acetate (10.60 g, 120 mmol) in anhydrous benzene (30 ml) was slowly added. The mixture was then refluxed for 4 h, cooled at 0 °C, treated with absolute ethanol (3 ml) and stirred for 10 min. After dilution with diethyl ether (100 ml), the mixture was poured into ice-water/glacial acetic acid 1:1 (50 ml), the organic layer separated and

the aqueous layer further extracted with diethyl ether. The combined organic extracts were washed with saturated sodium carbonate solution and water, dried (magnesium sulfate) and evaporated under reduced pressure to give a liquid residue, which was purified by bulb-to-bulb distillation in vacuo. IR spectra in $CHCl_3$ of all compounds showed absorption values (ν_{max}) for both ester and ketone functions, ranging from 1730 to 1738 cm^{-1} and from 1673 to 1687 cm^{-1}, respectively.

*4.1.2. General procedure for compounds **2a–i***

A solution of the corresponding aroylacetate **1a–i** (20 mmol) in *N*,*N*-dimethylformamide dimethyl acetal (2.86 g, 24 mmol) was refluxed for 1 h. The excess acetal was distilled off under reduced pressure and the residue was chromatographed on Florisil, using diethyl ether as eluant. The solid product was then recrystallized from anhydrous diethyl ether/petroleum ether.

*4.1.2.1. Ethyl 3-dimethylamino-2-(4-methoxybenzoyl)-acrylate **2f**.* Yield 91%; m.p. 74–75 °C; IR ($CHCl_3$), cm^{-1}: 1675, 1625, 1600; ^1H NMR ($CDCl_3$) δ: 0.98 (t, $J = 7.2$, 3H, ethyl CH_3), 2.96 [s, 6H, $N(CH_3)_2$], 3.87 (s,3H, CH_3O), 4.02 (q, $J = 7.2$, 2H, ethyl CH_2), 6.93 (d, $J = 8.4$, 2H, aromatic), 7.70 (s, 1H, =CH), 7.83 (d, $J = 8.4$, 2H, aromatic). *Anal.* $C_{15}H_{19}NO_4$ (C, H, N).

*4.1.2.2. Methyl 2-benzoyl-3-dimethylaminoacrylate **2i**.* Yield 72%; m.p. 83–84 °C; IR ($CHCl_3$), cm^{-1}: 1685, 1628, 1600; ^1H NMR ($CDCl_3$) δ: 2.96 [s, 6H, $N(CH_3)_2$], 3.50 (s, 3H, CH_3O), 7.25–7.6 (m, 3H, aromatic + =CH), 7.65–8.0 (m, 3H, aromatic). *Anal.* $C_{13}H_{15}NO_3$ (C, H, N).

*4.1.3. General procedure for compounds **3a–i,k–s***

[4-(Methylsulfonyl)phenyl]hydrazine hydrochloride (2.23 g, 10 mmol) (**3a–i**) or (4-hydrazinobenzenesulfonamide hydrochloride (2.24 g, 10 mmol) (**3k–s**) was added to a stirred solution of **2a–i** (10 mmol) in absolute ethanol (60 ml). The mixture was heated to reflux for 2 h and evaporated under reduced pressure. The residue was taken up in chloroform and the organic solution was washed with water, dried (magnesium sulfate), filtered and evaporated under reduced pressure. The solid residue was purified by recrystallization from 95% ethanol.

For the analytical, physical and spectral data of compounds **3a–i** see Tables 1 and 3; for **3k–s** see Tables 2 and 4.

Nitriles **3a**,**k** were obtained in a mixture with the corresponding amides from which they were separated by chromatography on silica gel: nitriles were eluted with ethyl acetate/petroleum ether (b.p. 40–60 °C) 1:1; amides, successively, with pure ethyl acetate.

The analytical, physical and spectral data of amides are below reported.

Amide corresponding to **3a**:

Yield 10%; m.p. 239–40 °C (from 95% ethanol); IR (KBr), cm^{-1}: 3450, 3390, 1628; ^1H NMR (DMSO-d_6) δ: 3.31 (s, 3H, CH_3), 7.47 (s, 2H, NH_2, exchange with D_2O), 7.5–7.65 (m, 3H, aromatic), 7.75–7.85 (m, 2H, aromatic), 7.91 (d, $J = 8.6$, 2H, aromatic), 7.92 (s, 1H, pyrazole H-3), 8.12 (d, $J = 8.6$, 2H, aromatic). *Anal.* $C_{17}H_{15}N_3O_3S$ (C, H, N, S).

Amide corresponding to **3k**:

Yield 8%; m.p. 212–213 °C (from 95% ethanol); IR (KBr), cm^{-1}: 3440, 3400, 3360, 3275, 1620; ^1H NMR (DMSO-d_6) δ: 7.42 (s, 2H, $CONH_2$, exchange with D_2O), 7.5–7.65 (m, 5H, aromatic + SO_2NH_2, 2H exchange with D_2O), 7.75–7.85 (m, 3H, aromatic + pyrazole H-3), 7.87 (d, $J = 8.8$, 2H, aromatic), 8.00 (d, $J = 8.8$, 2H, aromatic). *Anal.* $C_{16}H_{14}N_4O_3S$ (C, H, N, S).

*4.1.4. General procedure for compounds **3j,t***

A solution of **4g**,**p** (10 mmol) in anhydrous methanol (50 ml) added with concentrated sulphuric acid (6 drops) was refluxed with stirring for 14 h. After cooling to room temperature, the reaction mixture was diluted with water (30 ml), concentrated under reduced pressure and extracted with chloroform (3 × 30 ml). The extracts were washed twice with saturated sodium hydrogen carbonate solution and with water, dried (magnesium sulfate), filtered and evaporated in vacuo, to give a solid residue which was recrystallized from 95% ethanol.

For the analytical, physical and spectral data of compound **3j** see Tables 1 and 3; for **3t** see Tables 2 and 4.

*4.1.5. General procedure for compounds **4b–g,l–r***

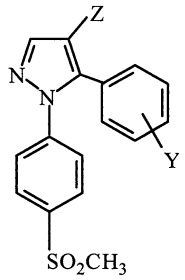
Potassium hydroxide (1.68 g, 30 mmol) dissolved in 95% ethanol (10 ml) was added to a solution of **3b–g**,**l–r** (10 mmol) in the same solvent (30 ml). The mixture was refluxed with stirring for 5 h, the solvent was evaporated under reduced pressure and the residue dissolved with water (50 ml). The aqueous solution was acidified with 6 N hydrochloric acid (pH ~1) and the white solid which separated was collected by filtration and washed with water. The crude product was then recrystallized from 95% ethanol.

For the analytical, physical and spectral data of compounds **4b–g**,**l–r** see Tables 5 and 6.

*4.1.6. General procedure for compounds **5l–q***

A solution of **3l–q** (10 mmol) in anhydrous tetrahydrofuran (60 ml) was slowly added to a stirred solution of lithium aluminum hydride (0.76 g, 20 mmol) in the same solvent (50 ml). The mixture was refluxed with stirring for 24 h, cooled at 0 °C, diluted with diethyl ether (100 ml) and treated in succession with water (2 ml), 10% sodium hydroxide solution (2 ml) and water (10 ml). The supernatant organic solution was decanted and the insoluble residue was treated with 3 N hydrochloric acid until an acid aqueous suspension was

Table 1
Structures and chemical data of nitrile (**3a**) and esters (**3b**–**j**)



Compound	Y	Z	Yield (%)	M.p. (°C) [a]	Molecular formula [b]
3a	H	CN	73	213–14	$C_{17}H_{13}N_3O_2S$
3b	H	$CO_2C_2H_5$	90	131–32	$C_{19}H_{18}N_2O_4S$
3c	4-F	$CO_2C_2H_5$	86	129–30	$C_{19}H_{17}FN_2O_4S$
3d	4-Cl	$CO_2C_2H_5$	95	156–57	$C_{19}H_{17}ClN_2O_4S$
3e	4-CH_3	$CO_2C_2H_5$	97	163–64	$C_{20}H_{20}N_2O_4S$
3f	4-OCH_3	$CO_2C_2H_5$	93	109–10	$C_{20}H_{20}N_2O_5S$
3g	3,4-$(OCH_3)_2$	$CO_2C_2H_5$	88	142–43	$C_{21}H_{22}N_2O_6S$
3h	4-NO_2	$CO_2C_2H_5$	97	166–67	$C_{19}H_{17}N_3O_6S$
3i	H	CO_2CH_3	78	192–93	$C_{18}H_{16}N_2O_4S$
3j	3,4-$(OCH_3)_2$	CO_2CH_3	65	188–89	$C_{20}H_{20}N_2O_6S$

[a] From 95% ethanol.
[b] All compounds were analysed for C, H, N, S.

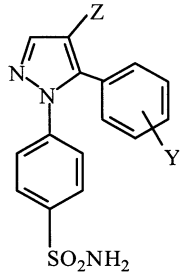
obtained (pH ~ 1). Insoluble white solid was collected by filtration and washed with water. The crude product was then recrystallized from ethyl acetate.

For the analytical, physical and spectral data of compounds **3l**–**q** see Tables 7 and 8.

4.1.7. General procedure for compounds *6m,o*

A solution of **5m**,**o** (10 mmol) in anhydrous acetonitrile (100 ml) was added to a stirred suspension of pyridinium chlorochromate (PCC) (3.23 g, 15 mmol) in the same solvent (50 ml). The mixture was stirred at

Table 2
Structures and chemical data of nitrile (**3k**) and esters (**3l**–**t**)



Compound	Y	Z	Yield (%)	M.p. (°C) [a]	Molecular formula [b]
3k	H	CN	72	192–93	$C_{16}H_{12}N_4O_2S$
3l	H	$CO_2C_2H_5$	93	186–87	$C_{18}H_{17}N_3O_4S$
3m	4-F	$CO_2C_2H_5$	84	187–88	$C_{18}H_{16}FN_3O_4S$
3n	4-Cl	$CO_2C_2H_5$	92	195–96	$C_{18}H_{16}ClN_3O_4S$
3o	4-CH_3	$CO_2C_2H_5$	96	178–79	$C_{19}H_{19}N_3O_4S$
3p	4-OCH_3	$CO_2C_2H_5$	91	158–59	$C_{19}H_{19}N_3O_5S$
3q	3,4-$(OCH_3)_2$	$CO_2C_2H_5$	88	223–24	$C_{20}H_{21}N_3O_6S$
3r	4-NO_2	$CO_2C_2H_5$	94	162–63	$C_{18}H_{16}N_4O_6S$
3s	H	CO_2CH_3	80	197–98	$C_{17}H_{15}N_3O_4S$
3t	4-OCH_3	CO_2CH_3	59	214–15	$C_{18}H_{17}N_3O_5S$

[a] From 95% ethanol.
[b] All compounds were analysed for C, H, N, S.

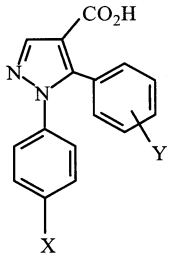
Table 3
Spectral data of compounds **3a–j**

Comp.	IR (cm^{-1}) ($CHCl_3$)	^1H NMR, δ ($CDCl_3$)
3a	2240 (CN); 1323, 1152 (SO_2)	3.08 (s, 3H, CH_3), 7.3–7.55 (m, 7H, aromatic), 7.94 (d, $J = 8.8$, 2H, aromatic), 8.07 (s, 1H, pyrazole H-3)
3b	1705 (CO); 1323, 1152 (SO_2)	1.22 (t, $J = 7.1$, 3H, ethyl CH_3), 3.04 (s, 3H, CH_3SO_2), 4.21 (q, $J = 7.1$, 2H, CH_2), 7.25–7.5 (m, 2H, aromatic), 7.85 (d, $J = 8.9$, 2H, aromatic), 8.22 (s, 1H, pyrazole H-3)
3c	1713 (CO); 1322, 1155 (SO_2)	1.24 (t, $J = 7.1$, 3H, ethyl CH_3), 3.05 (s, 3H, CH_3SO_2), 4.22 (q, $J = 7.1$, 2H, CH_2), 7.05–7.15 (m, 7H, aromatic), 7.25–7.35 (m, 2H, aromatic),, 7.41 (d, $J = 8.6$, 2H, aromatic), 7.88 (d, $J = 8.6$, 2H, aromatic), 8.21 (s, 1H, pyrazole H-3)
3d	1710 (CO); 1320, 1150 (SO_2)	1.25 (t, $J = 7.1$, 3H, ethyl CH_3), 3.05 (s, 3H, CH_3SO_2), 4.23 (q, $J = 7.1$, 2H, CH_2), 7.24 (d, $J = 8.5$, 2H, aromatic), 7.38 (d, $J = 8.6$, 2H, aromatic), 7.42 (d, $J = 8.8$, 2H, aromatic), 7.89 (d, $J = 8.6$, 2H, aromatic), 8.21 (s, 1H, pyrazole H-3)
3e	1705 (CO); 1320, 1152 (SO_2)	1.24 (t, $J = 7.1$, 3H, ethyl CH_3), 2.39 (s, 3H, CH_3Ar), 3.04(s, 3H, CH_3SO_2), 4.22 (q, $J = 7.1$, 2H, CH_2), 7.18 (s, 4H, aromatic), 7.42 (d, $J = 8.8$, 2H, aromatic), 7.86 (d, $J = 8.8$, 2H, aromatic), 8.20 (s, 1H, pyrazole H-3)
3f	1708 (CO); 1320, 1153 (SO_2)	1.26 (t, $J = 7.1$, 3H, ethyl CH_3), 3.05 (s, 3H, CH_3SO_2), 3.84 (s, 3H, CH_3O), 4.23 (q, $J = 7.1$, 2H, CH_2), 6.90 (d, $J = 8.8$, 2H, aromatic), 7.21 (d, $J = 8.8$, 2H, aromatic), 7.43 (d, $J = 8.6$, 2H, aromatic), 7.86 (d, $J = 8.6$, 2H, aromatic), 8.20 (s, 1H, pyrazole H-3)
3g	1710 (CO); 1322, 1153 (SO_2)	1.26 (t, $J = 7.1$, 3H, ethyl CH_3), 3.04 (s, 3H, CH_3SO_2), 3.76 (s, 3H, CH_3O), 3.91 (s, 3H, CH_3O), 4.24 (q, $J = 7.1$, 2H, CH_2), 6.75–6.9 (m, 3H, aromatic), 7.45 (d, $J = 8.6$, 2H, aromatic), 7.87 (d, $J = 8.6$, 2H, aromatic), 8.20 (s, 1H, pyrazole H-3)
3h	1718 (CO); 1525, 1350 (NO_2); 1323, 1157 (SO_2)	1.26 (t, $J = 7.1$, 3H, ethyl CH_3), 3.06 (s, 3H, CH_3SO_2), 4.24 (q, $J = 7.1$, 2H, CH_2), 7.41 (d, $J = 8.5$, 2H, aromatic), 7.52 (d, $J = 8.9$, 2H, aromatic), 7.91 (d, $J = 8.4$, 2H, aromatic), 8.25 (s, 1H, pyrazole H-3), 8.26 (d, $J = 8.9$, 2H, aromatic)
3i	1712 (CO); 1320, 1152 (SO_2)	3.09 (s, 3H, CH_3SO_2), 3.76 (s, 3H, CH_3OCO), 7.25–7.5 (m, 7H, aromatic), 7.85 (d, $J = 8.5$, 2H, aromatic), 8.21 (s, 1H, pyrazole H-3)
3j	1715 (CO); 1318, 1157 (SO_2)	3.04 (s, 3H, CH_3SO_2), 3.77 (s, 6H, 2 CH_3), 3.91 (s, 3H, CH_3), 6.75–6.9 (m, 3H, aromatic), 7.45 (d, $J =$ 8.6, 2H, aromatic), 7.87 (d, $J = 8.6$, 2H, aromatic), 8.20 (s, 1H, pyrazole H-3)

Table 4
Spectral data of compounds **3k–t**

Comp.	IR (cm^{-1}) (KBr)	^1H NMR, δ (DMSO-d_6)
3k	3375, 3240 (NH_2); 2230 (CN); 1352, 1168 (SO_2)	7.35–7.45 (m, 2H, aromatic), 7.5–7.6 (m, 7H, aromatic+NH_2, 2H exchange with D_2O), 7.87 (d, $J = 8.6$, 2H, aromatic), 8.51 (s, 1H, pyrazole H-3)
3l	3370, 3240 (NH_2); 1700 (CO); 1342, 1168 (SO_2)	1.13 (t, $J = 7.1$, 3H, CH_3), 4.14 (q, $J = 7.1$, 2H, CH_2), 7.3–7.45 (m, 7H, aromatic), 7.48 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J = 8.5$, 2H, aromatic), 8.27 (s, 1H, pyrazole H-3)
3m	3320, 3215 (NH_2); 1705 (CO); 1367, 1175 (SO_2)	1.15 (t, $J = 7.1$, 3H, CH_3), 4.14 (q, $J = 7.1$, 2H, CH_2), 7.2–7.35 (m, 2H, aromatic), 7.35–7.5 (m, 6H, aromatic+NH_2, 2H exchange with D_2O), 7.82 (d, $J = 8.9$, 2H, aromatic), 8.28 (s, 1H, pyrazole H-3)
3n	3340, 3225 (NH_2); 1705 (CO); 1365, 1172 (SO_2)	1.15 (t, $J = 7.1$, 3H, CH_3), 4.15 (q, $J = 7.1$, 2H, CH_2), 7.35–7.55 (m, 8H, aromatic+NH_2, 2H exchange with D_2O), 7.83 (d, $J = 8.5$, 2H, aromatic), 8.27 (s, 1H, pyrazole H-3)
3o	3370, 3230 (NH_2); 1685 (CO); 1342, 1163 (SO_2)	1.15 (t, $J = 7.1$, 3H, ethyl CH_3), 2.34 (s, 3H, CH_3Ar), 4.14 (q, $J = 7.1$, 2H, CH_2), 7.22 (s, 4H, aromatic), 7.42 (d, $J = 8.6$, 2H, aromatic), 7.48 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J =$ 8.6, 2H, aromatic), 8.25 (s, 1H, pyrazole H-3)
3p	3290, 3215 (NH_2); 1700 (CO); 1345, 1172 (SO_2)	1.17 (t, $J = 7.1$, 3H, ethyl CH_3), 3.79 (s, 3H, CH_3O), 4.15 (q, $J = 7.1$, 2H, CH_2), 6.96 (d, $J =$ 8.9, 2H, aromatic), 7.27 (d, $J = 8.8$, 2H, aromatic), 7.43 (d, $J = 8.8$, 2H, aromatic), 7.49 (s, 2H, NH_2, exchange with D_2O), 7.82 (d, $J = 8.6$, 2H, aromatic), 8.24 (s, 1H, pyrazole H-3)
3q	3360, 3265 (NH_2); 1697 (CO); 1340, 1160 (SO_2)	1.66 (t, $J = 7.1$, 3H, ethyl CH_3), 3.63 (s, 3H, CH_3O), 3.78 (s, 3H, CH_3O), 4.16 (q, $J = 7.1$, 2H, CH_2), 6.82 (dd, $J' = 8.8$, $J'' = 2$, 1H, aromatic), 6.9–7.0 (m, 2H, aromatic), 7.45 (d, $J = 8.5$, 2H, aromatic), 7.49 (s, 2H, NH_2, exchange with D_2O), 7.82 (d, $J = 8.5$, 2H, aromatic), 8.24 (s, 1H, pyrazole H-3)
3r	3320, 3250 (NH_2); 1727 (CO); 1523, 1335 (NO_2); 1345, 1177 (SO_2)	1.14 (t, $J = 7.1$, 3H, CH_3), 4.15 (q, $J = 7.1$, 2H, CH_2), 7.47 (d, $J = 8.8$, 2H, aromatic), 7.49 (s, 2H, NH_2, exchange with D_2O), 7.69 (d, $J = 8.8$, 2H, aromatic), 7.82 (d, $J = 8.6$, 2H, aromatic), 8.27 (d, $J = 8.8$, 2H, aromatic), 8.34 (s, 1H, pyrazole H-3)
3s	3360, 3265 (NH_2); 1700 (CO); 1343, 1167 (SO_2)	3.69 (s, 3H, CH_3), 7.3–7.45 (m, 7H, aromatic), 7.49 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J = 8.5$, 2H, aromatic), 8.29 (s, 1H, pyrazole H-3)
3t	3345, 3220 (NH_2); 1708 (CO); 1351, 1170 (SO_2)	3.77 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.91 (d, $J = 8.6$, 2H, aromatic), 7.21 (d, $J = 8.7$, 2H, aromatic), 7.26 (s, 2H, NH_2, exchange with D_2O), 7.38 (d, $J = 8.5$, 2H, aromatic), 7.85 (d, $J =$ 8.6, 2H, aromatic), 8.19 (s, 1H, pyrazole H-3)

Table 5
Structures and chemical data of carboxylic acids (**4b–g,l–r**)



Compound	X	Y	Yield (%)	M.p. (°C)	Molecular formula [e]
4b	SO_2CH_3	H	98	234–35 [a]	$C_{17}H_{14}N_2O_4S$
4c	SO_2CH_3	4-F	90	260–61 [b]	$C_{17}H_{13}FN_2O_4S$
4d	SO_2CH_3	4-Cl	83	253–54 [b]	$C_{17}H_{13}ClN_2O_4S$
4e	SO_2CH_3	4-CH_3	91	234–35 [b]	$C_{18}H_{16}N_2O_4S$
4f	SO_2CH_3	4-OCH_3	94	237–38 [a]	$C_{18}H_{16}N_2O_5S$
4g	SO_2CH_3	(3,4-OCH_3)$_2$	91	253–54 [b]	$C_{19}H_{18}N_2O_6S$
4l	SO_2NH_2	H	83	281–82 [b]	$C_{16}H_{13}N_3O_4S$
4m	SO_2NH_2	4-F	94	234–35 [a]	$C_{16}H_{12}FN_3O_4S$
4n	SO_2NH_2	4-Cl	94	255–56 [a]	$C_{16}H_{12}ClN_3O_4S$
4o	SO_2NH_2	4-CH_3	90	239–40 [c]	$C_{17}H_{15}N_3O_4S$
4p	SO_2NH_2	4-OCH_3	93	237–38 [c]	$C_{17}H_{15}N_3O_5S$
4q	SO_2NH_2	3,4-(OCH_3)$_2$	97	279–80 [d]	$C_{18}H_{17}N_3O_6S$
4r	SO_2NH_2	4-NO_2	93	262–63 [d]	$C_{16}H_{12}N_4O_6S\cdot H_2O$

[a] From ethyl acetate.
[b] From 95% ethanol.
[c] From ethyl acetate/petroleum ether (b.p. 40–70 °C).
[d] From anhydrous ethanol.
[e] All compounds were analysed for C, H, N, S.

room temperature for 24 h and, therefore, diluted with anhydrous diethyl ether (100 ml). The supernatant organic solution was decanted from a black gum, which was washed with anhydrous diethyl ether (3 × 30 ml). The combined organic solution was filtered on Florisil and the solvent was removed by distillation. The crude residue was purified by recrystallization from ethyl acetate.

*4.1.7.1. 4-[5-(4-Fluorophenyl)-4-formyl-1H-pyrazol-1-yl]benzenesulfonamide (**6m**).* Yield 81%; m.p. 179–180 °C; IR (KBr), cm^{-1}: 3305, 3170, 1677, 1345, 1163; ^1H NMR ($CDCl_3$) δ: 4.92 (s, 2H, NH_2, exchange with D_2O), 7.1–7.35 (m, 4H, aromatic), 7.41 (d, $J = 8.9$, 2H, aromatic), 7.91 (d, $J = 8.9$, 2H, aromatic), 8.27 (s, 1H, pyrazole H-3), 9.76 (s, 1H, CHO). *Anal.* $C_{16}H_{12}N_3O_3S$ (C, H, N, S).

*4.1.7.2. 4-[5-(4-methylphenyl)-4-formyl-1H-pyrazol-1-yl]benzenesulfonamide (**6o**).* Yield 53%; m.p. 207–209 °C; IR (KBr), cm^{-1}: 3345, 3290, 1655, 1357, 1168; ^1H NMR ($CDCl_3$) δ: 2.43 (s, 3H, CH_3), 4.89 (s, 2H, NH_2, exchange with D_2O), 7.15–7.3 (m, 4H, aromatic), 7.43 (d, $J = 8.6$, 2H, aromatic), 7.89 (d, $J = 8.6$, 2H, aromatic), 8.26 (s, 1H, pyrazole H-3), 9.74 (s, 1H, CHO). *Anal.* $C_{17}H_{15}N_3O_3S$ (C, H, N, S).

4.2. Pharmacology

All tested compounds were administered orally, at the initial dose of 200 mg/kg. Compounds, which exhibited a statistically significant activity at this dose, were further tested at the dose of 100 mg/kg.

4.2.1. Anti-inflammatory activity

The carrageenan-induced paw edema test [28] was used on groups of five rats. Sixty minutes after administering the test compound, 0.1 ml of a 1% carrageenan solution in saline was injected into the plantar surface of the right hind paw of each rat. Paw volume, as determined by measuring the amount of water displaced after immersing the paw up to the level of the lateral malleolus, was recorded immediately after the carrageenan injection and again 2 h later. The difference between these two values was taken as edema volume. The percent inhibition of the edema of treated rats with respect to controls was calculated. Indomethacin (6 mg/kg p.o.) was used as a reference standard.

Table 6
Spectral data of compounds **4b–g,l–r**

Comp.	IR (cm^{-1}) (KBr)	^1H NMR, δ (DMSO-d_6)
4b	3200–2400, 1675 (CO_2H)	3.26 (s, 3H, CH_3), 7.3–7.45 (m, 5H, aromatic), 7.47 (d, $J = 8.6$, 2H, aromatic), 7.92 (d, $J = 8.7$, 2H, aromatic), 8.26 (s, 1H, pyrazole H-3), ~12.2 (very br s, 1H, CO_2H, exchanges with D_2O)
4c	3200–2400, 1685 (CO_2H)	3.27 (s, 3H, CH_3), 7.2–7.35 (m, 2H, aromatic), 7.4–7.55 (m, 4H, aromatic), 7.94 (d, $J = 8.6$, 2H, aromatic), 8.25 (s, 1H, pyrazole H-3), 12.57 (br s, 1H, CO_2H, exchanges with D_2O)
4d	3200–2400, 1680 (CO_2H)	3.27 (s, 3H, CH_3), 7.35–7.55 (m, 6H, aromatic), 7.96 (d, $J = 8.6$, 2H, aromatic), 8.26 (s, 1H, pyrazole H-3), 12.59 (br s, 1H, CO_2H, exchanges with D_2O)
4e	3200–2400, 1683 (CO_2H)	2.40 (s, 3H, CH_3Ar), 3.05 (s, 3H, CH_3SO_2), 7.18 (s, 4H, aromatic), 7.41 (d, $J = 8.5$, 2H, aromatic), 7.87 (d, $J = 8.5$, 2H, aromatic), 8.25 (s, 1H, pyrazole H-3), 12.52 (br s, 1H, CO_2H, exchanges with D_2O)
4f	3200–2400, 1675 (CO_2H)	3.26 (s, 3H, CH_3SO_2), 3.79 (s, 3H, CH_3O), 6.96 (d, $J = 8.8$, 2H, aromatic), 7.27 (d, $J = 8.8$, 2H, aromatic), 7.48 (d, $J = 8.6$, 2H, aromatic), 7.94 (d, $J = 8.6$, 2H, aromatic), 8.22 (s, 1H, pyrazole H-3), ~12.4 (very br s, 1H, CO_2H, exchanges with D_2O)
4g	3200–2400, 1688 (CO_2H)	3.26 (s, 3H, CH_3SO_2), 3.60 (s, 3H, CH_3O), 3.78 (s, 3H, CH_3O), 6.84 (dd, $J' = 8.5$, $J'' = 2$, 1H, aromatic), 6.9–7.0 (m, 2H, aromatic), 7.50 (d, $J = 8.8$, 2H, aromatic), 7.94 (d, $J = 8.8$, 2H, aromatic), 8.22 (s, 1H, pyrazole H-3), ~12.4 (very br s, 1H, CO_2H, exchanges with D_2O)
4l	3200–2400, 1665 (CO_2H)	7.3–7.5 (m, 9H, aromatic+NH_2, 2H exchange with D_2O), 7.78 (d, $J = 8.6$, 2H, aromatic), 8.23 (s, 1H, pyrazole H-3), 12.49 (br s, 1H, CO_2H, exchanges with D_2O)
4m	3200–2400 1689 (CO_2H)	7.2–7.3 (m, 2H, aromatic), 7.35–7.55 (m, 6H, aromatic+NH_2, 2H exchange with D_2O), 7.81 (d, $J = 8.6$, 2H, aromatic), 8.23 (s, 1H, pyrazole H-3), ~12.5 (very br s, 1H, CO_2H, exchanges with D_2O)
4n	3200–2400, 1678 (CO_2H)	7.3–7.55 (m, 8H, aromatic+NH_2, 2H exchange with D_2O), 7.82 (d, $J = 8.7$, 2H, aromatic), 8.24 (s, 1H, pyrazole H-3), ~12.5 (br s, 1H, CO_2H, exchanges with D_2O)
4o	3200–2400, 1688 (CO_2H)	2.34 (s, 3H, CH_3), 7.24 (s, 4H, aromatic), 7.41 (d, $J = 8.7$, 2H, aromatic), 7.47 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J = 8.7$, 2H, aromatic), 8.21 (s, 1H, pyrazole H-3), 12.43 (br s, 1H, CO_2H, exchanges with D_2O)
4p	3200–2400, 1694 (CO_2H)	3.79 (s, 3H, CH_3O), 6.96 (d, $J = 8.8$, 2H, aromatic),7.26 (d, $J = 8.8$, 2H, aromatic), 7.42 (d, $J = 8.7$, 2H, aromatic), 7.48 (s, 2H, NH_2, exchange with D_2O), 7.81 (d, $J = 8.7$, 2H, aromatic), 8.20 (s, 1H, pyrazole H-3), 12.44 (br s, 1H, CO_2H, exchanges with D_2O)
4q	3200–2400, 1681 (CO_2H)	3.61 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 6.83 (dd, $J' = 8.5$, $J'' = 2$, 1H, aromatic), 6.9–7.0 (m, 2H, aromatic), 7.43 (d, $J = 8.5$, 2H, aromatic), 7.47 (s, 2H, NH_2, exchange with D_2O), 7.81 (d, $J = 8.5$, 2H, aromatic), 8.19 (s, 1H, pyrazole H-3), ~12.4 (very br s, 1H, CO_2H, exchanges with D_2O)
4r	3200–2400, 1705 (CO_2H)	7.45 (d, $J = 8.8$, 2H, aromatic), 7.48 (s, 2H, NH_2, exchanges with D_2O), 7.68 (d, $J = 8.9$, 2H, aromatic), 7.82 (d, $J = 8.6$, 2H, aromatic), 8.25 (d, $J = 8.9$, 2H, aromatic), 8.29 (s, 1H, CH-3 pyrazole), ~12.7 (very br s, 1H, CO_2H, exchanges with D_2O)

4.2.2. Analgesic activity

The writhing test [29] was used on a group of five mice. One hour after the administration of the test compound, 0.01 ml/g of a 0.6% acetic acid solution was injected intraperitoneally in each mouse. The writhing movements of each animal were counted for 10 min (between the 5th and 15th min after the injection of the irritant). The antinociceptive effect was expressed as the percentage of protection compared to the control group. Indomethacin (6 mg/kg p.o.) was used as a reference standard.

4.2.3. Platelet aggregation

Human blood samples from normal subjects were drawn through a 19-gauge needle, avoiding carefully prolonged venous stasis. None of these subjects was treated with any drug known to influence the platelet function.

Blood was collected in plastic tubes containing 3.8% trisodium citrate aqueous solution. Platelet rich plasma (PRP) was obtained by centrifuging the blood at $100 \times g$ for 20 min. Platelet poor plasma (PPP) was obtained by centrifuging the remaining blood at $1100 \times g$ for 15 min.

Platelet count in PRP was maintained at 300.000/mmc.

Platelet aggregation, performed in a Aggrecorder II PA 3220 aggregometer (Menarini, Firenze, Italy) was measured according to the Born's turbidimetric method [30] and quantified by the maximal light transmission reached 5 min after the addition of the agonist.

A first sample of PRP was pre-incubated at 37 °C for 2 min. After this time, platelet aggregation was induced adding adenosine diphosphate (ADP; 2 and 5 μM), collagen (4 and 8 μg/ml) or adrenaline (5 μM). A second sample of PRP was incubated for 2 min with a solution of the tested compound, before the addition of the agonist.

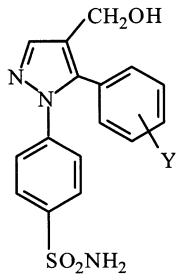
Comparing the maximal light transmission of the aggregation curves obtained with and without the addition of the tested compound, the percentage of inhibition of platelet aggregation was calculated.

4.3. Computational methods

4.3.1. Molecular docking

The crystal structures of murine COX-2 in complex with SC-558 (1cx2) [17] and ovine COX-1 in complex

Table 7
Structures and chemical data of carbinols (**5l**–**q**)



Compound	Y	Yield (%)	M.p. (°C)	Molecular formula [c]
5l	H	82	209–10 [a]	$C_{16}H_{15}N_3O_3S$
5m	4-F	85	204–05 [a]	$C_{16}H_{14}FN_3O_3S$
5n	4-Cl	88	184–85 [b]	$C_{16}H_{14}ClN_3O_3S$
5o	4-CH_3	84	181–83 [b]	$C_{17}H_{17}N_3O_3S$
5p	4-OCH_3	73	167–68 [a]	$C_{17}H_{17}N_3O_4S$
5q	3,4-$(OCH_3)_2$	58	228–30 [b]	$C_{18}H_{19}N_3O_5S$

[a] From 95% ethanol.
[b] From ethyl acetate.
[c] All compounds were analysed for C, H, N, S.

with flurbiprofen (1eqh) [31] were obtained from the Protein Data Bank. The potential of the 3D structures of COX-2 and COX-1 was assigned according to the Amber 4.0 force field.

The structural models of selected compounds, generated and energy minimized within MacroModel [32], were docked to COX-2 and COX-1 active sites using AutoDock 3.0 [33]. The region of interest used by Autodock was defined in such a way to include a specific portion of the catalytic site of the enzyme, in particular the system size was reduced for the docking calculations by including only residues containing atoms within 15 Å from any ligand atom. The search was carried out with the Lamarckian Genetic Algorithm: populations of 250 individuals with a mutation rate of 0.03 have been evolved for 100 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy, internal strain energy of the ligand, van der Waals and electrostatic interaction energies. All selected complexes underwent to a final energy minimization, before the model would be achieved. All calculations were performed on an O2 SGI workstation.

5. Results and discussion

In the carrageenan-induced paw edema test, at a dose of 200 mg/kg, only esters **3g**,**p** and nitrile **3k** showed a good anti-inflammatory activity, comparable to that of celecoxib, but in the writhing test they did not show the same analgesic effect as the reference drug (Table 9).

Considering the couples of nitriles **3a**,**k** and esters **3e**,**o**, pharmacological results confirmed a better antiphlogistic activity in vivo for sulfamoyl group with respect to methylsulfonyl one, as already observed [10], but, in the case of esters **3g**,**q**, the biological data did not match with these remarks.

As regards the ethyl esters **3g**,**p**, they appeared most effective as antiinflammatory agents than the corresponding methyl esters **3j**,**t**, but less active as analgesics.

Conversion of the ester group of **3g**,**p** into the carboxylic function (compounds **4g**,**p**) produced the

Table 8
Spectral data of compounds **5l**–**q**

Comp.	IR (cm^{-1}) (KBr)	^1H NMR, δ (DMSO-d_6)
5l	3480 (OH)	4.33 (d, $J = 5$, 2H, CH_2), 5.07 (t, $J = 5$, 1H, OH, exchanges with D_2O), 7.2–7.3 (m, 3H, aromatic), 7.34 (d, $J = 8.8$, 2H, aromatic), 7.4–7.5 (m, 4H, aromatic + NH_2, 2H exchange with D_2O), 7.79 (d, $J = 8.8$, 2H, aromatic), 7.88 (s, 1H, pyrazole H-3)
5m	3525 (OH)	4.31 (d, $J = 5$, 2H, CH_2), 5.08 (t, $J = 5$, 1H, OH, exchanges with D_2O), 7.25–7.35 (m, 4H, aromatic), 7.39 (d, $J = 8.6$, 2H, aromatic), 7.45 (s, 2H, NH_2, exchange with D_2O), 7.81 (d, $J = 8.6$, 2H, aromatic), 7.87 (s, 1H, pyrazole H-3)
5n	3485 (OH)	4.32 (d, $J = 5$, 2H, CH_2), 5.10 (t, $J = 5$, 1H, OH, exchanges with D_2O), 7.32 (d, $J = 8.6$, 2H, aromatic), 7.40 (d, $J = 8.6$, 2H, aromatic), 7.46 (s, 2H, NH_2, exchange with D_2O),7.53 (d, $J = 8.6$, 2H, aromatic), 7.82 (d, $J = 8.6$, 2H, aromatic), 7.88 (s, 1H, pyrazole H-3)
5o	3470 (OH)	2.35 (s, 3H, CH_3), 4.31 (d, $J = 5$, 2H, CH_2), 5.05 (t, $J = 5$, 1H, OH, exchanges with D_2O), 7.17 (d, $J = 8$, 2H, aromatic), 7.25 (d, $J = 8$, 2H, aromatic), 7.38 (d, $J = 8.5$, 2H, aromatic), 7.45 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J = 8.5$, 2H, aromatic), 7.85 (s, 1H, pyrazole H-3)
5p	3465 (OH)	3.80 (s, 3H, CH_3), 4.30 (d, $J = 5$, 2H, CH_2), 5.30 (t, $J = 5$, 1H, OH, exchanges with D_2O), 7.00 (d, $J = 9$, 2H, aromatic), 7.22 (d, $J = 9$, 2H, aromatic), 7.39 (d, $J = 8.8$, 2H, aromatic), 7.44 (s, 2H, NH_2, exchange with D_2O), 7.80 (d, $J = 8.9$, 2H, aromatic), 7.84 (s, 1H, pyrazole H-3)
5q	3470 (OH)	3.61 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 4.34 (d, $J = 5$, 2H, CH_2), 5.05 (t, $J = 5$, 1H, OH, exchanges with D_2O), 6.80 (dd, $J' = 8.3$, $J'' = 2$, 1H, aromatic), 6.88 (d, $J = 2$, 1H, aromatic), 7.01 (d, $J = 8.3$, 1H, aromatic), 7.41(d, $J = 9$, 2H, aromatic), 7.44 (s, 2H, NH_2, exchange with D_2O), 7.81 (d, $J = 9$, 2H, aromatic), 7.84 (s, 1H, pyrazole H-3)

Table 9
Anti-inflammatory and analgesic activities of tested compounds

Compound	Tested dose (mg/kg p.o.)	Anti-inflammatory activity in rat [a]	Analgesic activity in mice [b]
		Inhibition (%)	Protection (%)
3a	200	26	
3b	200	9	
3e	200	0	
3g	200	40**	7
	100	0	
3j	200	24	20
3k	200	46*	26
	100	30	
3o	200	21	
3p	200	37**	23
	100	15	
3q	200	23	
3t	200	25	37**
	100		31**
4g	200	0	
4p	200	14	
5m	200	0	
5n	200	20	
5o	200	16	
5q	200	17	
6o	200	24	
Celecoxib	200	45*	77**
Indomethacin	6	65*	85*
Dipyrone	100		58**
Morphine	15		84*

[a] Carrageenan-induced paw edema test ($n = 5$). Statistical significance *versus* control group ($n = 25$; control value: 215 ± 65) was evaluated by *t*-test.
[b] Writhing test ($n = 5$). Statistical significance versus control group ($n = 10$; control value: 52 ± 13) was evaluated by the Mann–Whitney test.
* $P < 0.01$.
** $P < 0.05$ versus control.

total loss or a strong decrease of anti-inflammatory activity; reduction of esters **3o**,**q** to alcohols **5o**,**q** and oxidation of **5o** to aldehyde **6o** did not produce any improvement in biological properties.

All carboxylic acids (**4b**–**g**,**l**–**r**), tested in vitro, as sodium salts, in platelet anti-aggregating assays, showed a strong inhibition of the platelet function (generally 70–90% at a concentration of 1×10^{-3} M), when ADP (5 or 2 μM) was used; good inhibition was also produced in the presence of collagen at a concentration of 1 μg/ml, whereas weak or no activity appeared when adrenaline (5 μM) or collagen (4 μg/ml) were employed as agonists (Table 10). The only isoform detectable in the platelets is COX-1, responsible of the production of thromboxane A_2 (TXA_2), a potent pro-aggregatory substance [34]; therefore, the inhibition of platelet aggregation could be acquired as an indirect proof of activity versus COX-1. The lack of anti-inflammatory efficacy together with good platelet anti-aggregating activity, as found for compounds **4g**,**p**, seems to confirm that the presence of a carboxylic function promotes selectivity for COX-1 [18].

In order to further rationalize these results, a molecular docking study was carried out on compounds **3g**,**k**, the most active as anti-inflammatory agents, and **4e**, endowed with interesting platelet anti-aggregating properties. Selected compounds were docked into the active site of COX-1 and COX-2, using AutoDock procedure. The ability of this program in predicting a conformation of the ligand very close to its X-ray structure has been widely described in literature [15].

Starting point for this study were the X-rays structures of murine COX-2 complexed with the analogue of celecoxib SC-558 (Fig. 3) [17] and ovine COX-1 complexed with the non-selective cyclooxygenase inhibitor flurbiprofen (Fig. 3) [31].

Concerning results on binding to COX-2, the main conformational difference between our 1,5-diarylpyrazoles and SC-558 is represented by the two torsional angles defined by rotation of the two phenyl rings on positions 1 and 5, respectively. According to our calculations (Fig. 4), 1,5-diarylpyrazoles are located in the centre of the COX-2 binding pocket in a conformation similar to the one adopted by SC-558 in the co-

Table 10
Platelet anti-aggregating activity of compounds **4b–g,l–r**: inhibition (%) of platelet aggregation induced by ADP, collagen and adrenaline in human plasma [a]

Comp.	Inhibition (%) of platelet aggregation induced by ADP, collagen and adrenaline in human plasma [a]				
	ADP (5 μM)	ADP (2 μM)	Collagen (4 μg/ml)	Collagen (1 μg/ml)	Adrenaline (5 μM)
4b	62	80	1	40	14
4c	79	82	2	27	0
4d	88	82	1	63	17
4e	76	81	9	84	56
4f	80	80	5	52	20
4g	73	10	6	40	5
4l	73	86	12	67	17
4m	81	78	15	14	3
4n	86	69	4	22	12
4o	68	81	18	76	13
4p	78	70	2	77	21
4q	7	90	0	30	7
4r	77	71	0	25	4
ASA	33	45	60	79	67

[a] Mean value of four determinations at the final concentration of tested compounds = 1×10^{-3} M.

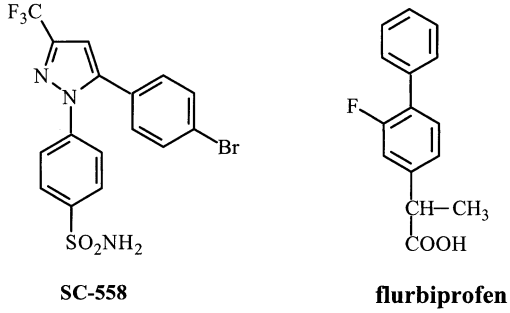
crystallized structure with this enzyme. They share with SC-558 a common hydrogen bonding interaction, Arg513, but lack the hydrogen bond with Arg120, being unsubstituted on position 3. The fluorine atom in the trifluoromethyl group of SC-558, in fact, acts as an acceptor to form a H-bond with one of the two NH_2 groups of Arg120 side chain, anchoring the inhibitor in the binding pocket. Compound **4e** is, however, able to form an additional hydrogen bond with the backbone carbonyl group of Ala527, in agreement with recent findings of Liu [15]; while **3k** shows an additional bond *via* a polar interaction between the ciano group on position 4 and the backbone carbonyl group of the same residue, Ala527. This interaction with Ala527, however, does not seem so strong as the one with the residue Arg120. Interestingly, the binding model obtained with AutoDock for **3g** points out that the substituent on position 4 should not exceed the ethoxycarbonyl group in order to maintain the favourable interaction with the active site of COX-2. In Fig. 4 is displayed the fitting of ester portion of **3g** in a small pocket defined by Ala527, Pro528, Ser530, Leu 531, Leu384, in agreement with the above cited study of Liu.

In the case of COX-1 complexes, the results of our calculations suggest that, among the studied molecules, carboxylic acid **4e** is able to interact with the enzyme active site in a way similar to flurbiprofen, the co-crystallized ligand for ovine COX-1, since it makes two hydrogen bonds with Arg120 (Fig. 5). The cyano derivative **3k** shows a common conformational preference as **4e**, but interacts with Arg120 making only a polar interaction. For ester **3g**, a different docking pose in the active site of COX-1 has been observed, probably due to the possibility of establishing a high number of hydrogen bonds with surrounding residues Val119, Ser121, Glu524, Leu531.

Considering these preliminary partial data from the biological tests and the computational studies, we could say therefore that these results are generally in agreement with the information already acquired on the SAR of diarylpyrazole cyclooxygenase inhibitors.

In particular, our modeling study:

i) confirms that the main interaction of COX-2 inhibitors with the enzyme is the hydrogen bond with Arg513, made by the sulfonamide or methylsulfone mojety, which has however the possibility of making an extended net of alternative hydrogen bonds in the surrounding area, interacting with residues Gln 192, Leu 352, Ser 353;
ii) suggests that the interaction with Arg120 could be important not only for COX-1, but also for COX-2 inhibitors, since our derivatives, which are



SC-558 **flurbiprofen**

Fig. 3. Structures of a selective COX-2 inhibitor (SC-558) and a non-selective COX inhibitor (flurbiprofen).

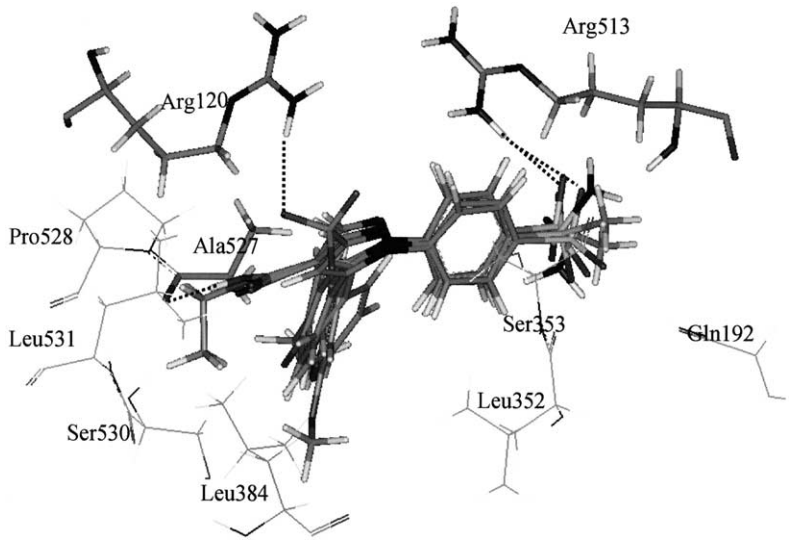


Fig. 4. Overlay of the docked orientations for **3g**,**k**, **4e** and SC-558 in the active site of COX-2. The most significative amino acid residues are reported and labelled accordingly. Residues involved in hydrogen bonds with the inhibitors are represented as sticks, the dashed lines are the hydrogen bonds.

not able to make an hydrogen bond with this residue, result less potent than celecoxib and SSC-558;

iii) confirms that substituents on position 4 are able to interact with Ala527, but should not exceed a well defined volume. Among the groups introduced by us in this position, the ethoxycarbonyl is the best in fitting the small hydrophobic pocket defined by residues Ala527, Pro528, Ser530, Leu531, Leu384,. However, none of the substituents we have introduced on position 4 of the pyrazole ring produced an improvement of the anti-inflammatory activity in comparison with celecoxib.

Further biological and 3D QSAR investigations are in progress to provide possible guidelines and suggestions for the rational design of improved COX-1 or COX-2 inhibitors.

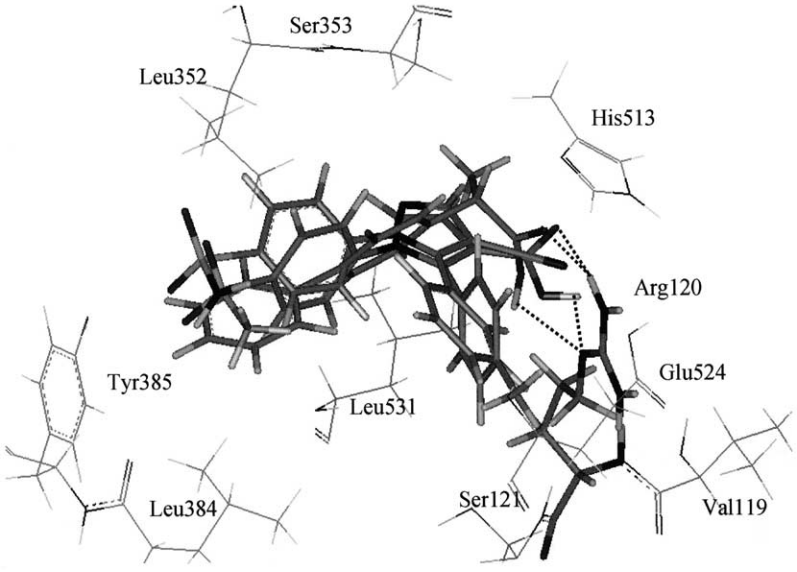


Fig. 5. Overlay of the docked orientations for **3k**, **4e** and flurbiprofen in the active site of COX-1. The most significative amino acid residues are reported and labelled accordingly. Residues involved in hydrogen bonds with the inhibitors are represented as sticks, the dashed lines are the hydrogen bonds.

Acknowledgements

We would like to thank the Ministero dell'Università e della Ricerca Scientifica e Tecnologica for the financial support.

References

[1] J.J. Talley, Selective inhibitors of cyclooxygenase-2 (COX-2), in: F.D. King, A.W. Oxford (Eds.), Progress in Medicinal Chemistry, vol. 36, Elsevier, Amsterdam, 1999, pp. 201–234 (and references cited therein).
[2] L.R. Howe, A.J. Dannenberg, A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer, Semin. Oncol. 29 (2002) 111–119.
[3] D. Slater, W. Dennes, R. Sawdy, V. Allport, P. Bennet, Expression of cyclo-oxygenase types-1 and -2 in human fetal membranes throughout pregnancy, J. Mol. Endocrinol. 22 (1999) 125–130.
[4] P.S. Aisen, Evaluation of selective COX-2 inhibitors for the treatment of Alzheimer's disease, J. Pain Symptom Manage. 23 (2002) S35–40.
[5] J.S. Guo, C.H. Cho, E.S. Lam-Liu, H.T. Choy, J.Y. Wang, M.W. Leung-Koo, Antiangiogenic effect of a highly selective cyclooxygenase-2 inhibitor on gastric ulcer healing in rats, Toxicol. Appl. Pharmacol. 183 (2002) 41–45.
[6] G. Dannhardt, W. Kiefer, G. Krämer, S. Maehrlein, U. Nowe, B. Fiebich, The pyrrole moiety as a template for COX-1/COX-2 inhibitors, Eur. J. Med. Chem. 35 (2000) 499–510.
[7] B. Hinz, K. Brune, Specific COX-2 inhibitors. Basis and options of a pharmacotherapeutical concept, Anaesthesist. 49 (2000) 964–971.
[8] H. Hashimoto, K. Imamura, J. Haruta, K. Wakitani, 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. 45 (2002) 1511–1517.
[9] R.W. McMurray, K.J. Hardy, Cox-2 inhibitors: today and tomorrow, Am. J. Med. Sci. 323 (2002) 181–189.
[10] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, P.C. Isakson, Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib), J. Med. Chem. 40 (1997) 1347–1365.
[11] P. Prasit, Z. Wang, C. Brideau, C.C. Chan, S. Charleson, W. Cromlish, D. Ethier, J.F. Evans, A.W. Ford-Hutchinson, J.Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Léger, J. Mancini, G.P. O'Neill, M. Ouellet, M.D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Thérien, P. Vickers, E. Wong, L.J. Xu, R.N. Young, R. Zamboni, The discovery of rofecoxib, [MK 966, Vioxx®, 4-(4′-methylsulfonylphenyl)-3-phenyl-2(5*H*)-furanone], an orally active cyclooxygenase-2 inhibitor, Biorg. Med. Chem. Lett. 9 (1999) 1773–1778.
[12] G. Menozzi, L. Mosti, L. Merello, A. Piana, U. Armani, M. Ghia, M. Angiola, F. Mattioli, 4-Dialkylamino-1-(5-substituted or unsubstituted 1-phenyl-1*H*-pyrazol-4-yl)butan-1-ols: synthesis and evaluation of analgesic, anti-inflammatory and platelet anti-aggregating activities, Farmaco 55 (2000) 219–226.
[13] D.B. Reitz, J.J. Li, M.B. Norton, E.J. Reinhard, J.T. Collins, G.D. Anderson, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, P.C. Isakson, Selective cyclooxygenase inhibitors: novel 1,2-diarylcyclopentenes are potent and orally active COX-2 inhibitors, J. Med. Chem. 37 (1994) 3878–3881.
[14] I.K. Khanna, R.M. Weiner, Y. Yu, X.D. Xu, F.J. Koszyk, P.W. Collins, C.M. Koboldt, A.W. Veenhuizen, W.E. Perkins, J.J. Casler, J.L. Masferrer, Y.Y. Zhang, S.A. Gregory, K. Seibert, P.C. Isakson, 1,2-Diarylimidazoles as potent, cyclooxygenase-2 selective, and orally active antiinflammatory agents, J. Med. Chem. 40 (1997) 1634–1647.
[15] H. Liu, X. Huang, J. Shen, X. Luo, M. Li, B. Xiong, G. Chen, J. Shen, Y. Yang, H. Jiang, K. Chen, Inhibitory mode of 1,5-diarylpyrazole derivatives against cyclooxygenase-2 and cyclooxygenase-1: molecular docking and 3D QSAR analyses, J. Med. Chem. 45 (2002) 4816–4827.
[16] A.S. Kalgutkar, A.B. Marnett, B.C. Crews, R.P. Remmel, L.J. Marnett, Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors, J. Med. Chem. 43 (2000) 2860–2870.
[17] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Pennig, K. Seibert, P.C. Isakson, W.C. Stallings, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (1996) 644–648.
[18] J.J. Talley, D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel, K. Seibert, 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, Valdecoxib: a potent and selective inhibitor of COX-2, J. Med. Chem. 43 (2000) 775–777.
[19] G. Menozzi, L. Mosti, P. Schenone, Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. VI. Synthesis of ethyl or methyl 1,5-disubstituted 1*H*-pyrazole-4-carboxylates, J. Heterocyclic Chem. 24 (1987) 1669–1675.
[20] G. Menozzi, P. Schenone, L. Mosti, F. Mattioli, Synthesis of 5-substituted 1-aryl-1*H*-pyrazole-4-acetonitriles, 4-methyl-1-phenyl-1*H*-pyrazole-3-carbonitriles and pharmacologically active 1-aryl-1*H*-pyrazole-4-acetic acids, J. Heterocyclic Chem. 30 (1993) 997–1002.
[21] G. Menozzi, L. Mosti, P. Fossa, F. Mattioli, M. Ghia, ω-Dialkylaminoalkyl ethers of phenyl(5-substituted 1-phenyl-1*H*-pyrazol-4-yl)methanols with analgesic and anti-inflammatory activity, J. Heterocyclic Chem. 34 (1997) 963–968.
[22] S. Morimura, Formation of 3,5-dicyano-4-(*N*,*N*-dimethylformamidino)-2,6-diphenyl-4*H*-pyran from ω-cyanoacetophenone, Heterocycles 14 (1980) 1449–1454.
[23] E.J. Breaux, K.E. Zwikelmaier, An improved general synthesis of 4-aryl-5-pyrimidinecarboxylates, J. Heterocyclic Chem. 18 (1981) 183–184.
[24] H.W. Gschwend, Pyrazolobenzazepines, US 4 028 381 (1977); Chem. Abstr. 87 (1977) 117854u.
[25] J.E. Franz, D.E. Schafer, L.H. Brannigan, Herbicidal compositions containing 5-aryl-4-isoxazolecarboxylate, Belg. 880 849 (1980); Chem. Abstr. 94 (1981) 78442f.
[26] R. Pepin, C. Schmitz, G.B. Lacroix, P. Dellis, C. Veyrat, (Morpholinocarbonyl)benzothiophenes and analogs as agrochemical fungicides and their preparation, Eur. Pat. Appl. EP 360 701 (1990); Chem. Abstr. 113 (1990) 78409m.
[27] A. Kuno, Y. Sugiyama, K. Katsuta, H. Sakai, H. Takasugi, Studies on cerebral protective agents. II. Novel 4-arylpyrimidine derivatives with anti-anoxic and anti-lipid peroxidation activities, Chem. Pharm. Bull. 40 (1992) 2423–2431.
[28] C.A. Winter, E.A. Risley, G.W. Nuss, Anti-inflammatory and antipyretic activities of indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid, J. Pharmacol. Exp. Ther. 141 (1963) 369–376.

[29] R. Koster, M. Anderson, E.J. De Beer, Synthetic analgesics. Dithienbutenyl dithienbuthylamines, Fed. Proc. 18 (1959) 412–421.
[30] G.V.R. Born, Aggregation of blood platelets by adenosine diphosphate and its reversal, Nature 194 (1962) 927–929.
[31] D. Picot, P.J. Loll, R.M. Garavito, The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1, Nature 367 (1994) 243–249.
[32] F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W.C. Still, MacroModel—an integrated software system for modeling organic and bioorganic molecules using molecular mechanics, J. Comput. Chem. 11 (1990) 440–467.
[33] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639–1662.
[34] J.R. Vane, Y.S. Bakhle, R.M. Botting, Cyclooxygenase 1 and 2, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 97–120.